Azotaemic renal osteodystrophy: a quantitative study on iliac bone

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SYNOPSIS The histopathology of bone is described in 60 patients with chronic renal failure due to a variety of renal diseases. Changes of azotaemic renal osteodystrophy included osteitis fibrosa, osteomalacia, and osteosclerosis. Quantitative histology using a point-counting technique revealed a significant increase in total bone, mineralized bone, and osteoid in comparison with a control group of 68 individuals. Osteitis fibrosa due to secondary hyperparathyroidism occurred in 93%, osteomalacia in 40%, and osteosclerosis in 30% of patients. Woven bone formation was a characteristic feature and was related to the severity of osteitis fibrosa. There were significant correlations between the weights of parathyroid glands and the number of osteoclasts, amounts of woven bone, and marrow fibrosis in the ilium. Hyperparathyroidism caused degradation of mineralized bone but the loss was balanced or exceeded by the aggradation of woven mineralized bone. Woven bone formation together with excess osteoid gave rise to osteosclerosis. The histological findings indicate that hyperparathyroidism and osteitis fibrosa usually occur early in chronic renal failure and that osteomalacia develops subsequently.

Chronic renal failure may be accompanied by bone disease (Stanbury, 1957, 1972) and the changes, which include osteitis fibrosa due to secondary hyperparathyroidism, osteomalacia and osteosclerosis, may conveniently be termed renal osteodystrophy (Liu and Chu, 1943). Although it is recognized that the clinical effects of such changes may be severe in childhood (Claireaux, 1953; Haust, Landing, Holmstrand, Currarino, and Smith, 1964), in adults they are seldom marked and symptoms attributable to bone disease are usually overshadowed by those of renal failure. Since the introduction of intermittent haemodialysis the lives of patients with chronic renal failure have been prolonged and symptoms of bone disease now constitute a major clinical problem in adults in some dialysis centres. In Newcastle upon Tyne dialysed patients commonly develop bone pain and pathological fractures, for example, in the ribs, metacarpals, and vertebrae (Kerr, Walls, Ellis, Simpson, Uldall, and Ward, 1969; Siddiqui and Kerr, 1971). As part of a wider clinical, radiological, and pathological study of bone changes in these patients, during the last six years we have examined bone from more than 200 dialysed and non-dialysed patients with renal failure. Early in this study it appeared that there were histological differences between the bone changes in dialysed and non-dialysed patients. The latter tended to show osteitis fibrosa, osteomalacia, and a normal or raised total bone mass with normal or elevated amounts of mineralized bone. In contrast, in the bones of dialysed patients there was a loss of mineralized lamellar bone and total bone after about one or two years on dialysis. In others, following more prolonged dialysis, the total bone mass was elevated due to excess osteoid formation whilst the amount of mineralized bone remained low. The bones of dialysed patients in general also showed less severe changes of osteitis fibrosa (Ellis and Peart, 1971).

To determine whether or not these differences were real it became necessary to quantitate the amounts of mineralized bone and osteoid present and the severity of the osteitis fibrosa. Critical quantitative histological studies of the mineralized and non-mineralized bone in renal failure were lacking until Garner and Ball (1966) reported their findings in 42 controls and in 18 selected cases of azotaemic renal osteodystrophy using a point-counting technique to study undecalcified sections of iliac crest. As a basis for our quantitative observations in renal failure we have similarly studied the

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iliac bone from 68 control subjects (Ellis and Peart, 1972). In this paper we present our findings in 60 patients with renal failure not treated by intermittent haemodialysis; the results in dialysed patients will be reported later. The main purposes of the present investigation were: (1) to define the range of bone changes which occur in azotaemic renal failure to form a basis for comparison with the bone changes in dialysed patients; (2) to examine the incidence and extent of woven bone formation; and (3) to determine the relative incidences of osteitis fibrosa, osteosclerosis, and osteomalacia. It was hoped that this might also throw some light on the existing controversy regarding the relative times of onset of hyperparathyroidism and osteomalacia in renal failure.

Materials and Methods

BONE

Samples of iliac bone were obtained by translilial biopsy (26 specimens) or at necropsy (37 specimens) from 60 patients with chronic renal failure. The biopsy specimens were obtained by our colleagues Drs J. Walls and J. Siddiqui using a trephine with an internal diameter of 0.7 cm (Byers and Smith, 1967). The amounts of mineralized and non-mineralized bone and the widths of osteoid seams were determined in undecalcified sections as previously described (Ellis and Peart, 1972). In most cases coming to necropsy additional bones such as ribs, clavicle, and vertebra were examined histologically. The calcification front was studied in all cases using toluidine blue or haematoxylin-stained sections (Raina, 1972). In 22 patients at necropsy the calcification front was also studied by the lipid-staining technique (fig 1) of Irving using Sudan Black (Irving, 1958, 1959).

Assessment of Osteitis Fibrosa

The severity of osteitis fibrosa involving compact and cancellous bone in decalcified sections of the ilium was assessed on a semi-quantitative scale from 0 to 5, taking into account the extent of active osteoclastic resorption of bone and the amount of marrow fibrosis. Briefly, in grade 1 osteitis fibrosa there was scant focal surface resorption of bone by occasional multinucleated osteoclasts associated with marrow condensation or early fibrosis; in grade 2 cases there was more widespread and conspicuous superficial and deep osteoclastic resorption of bone with marrow fibrosis; in grade 3 osteitis fibrosa the changes were more marked with extensive surface resorption and tunnelling by osteoclasts and marrow fibrosis extending around much of the trabecular surface. In grades 4 and 5 osteitis fibrosa there was progressively more bone resorption, often with collections of multinucleated osteoclasts and extensive areas of confluent marrow fibrosis. With practice it was found possible to allocate to individual cases an osteitis fibrosa rating at half unit intervals.

Osteoclasts, Woven Bone, and Marrow Fibrosis

The number of multinucleated osteoclasts in cancellous bone was determined in decalcified sections of ilium and expressed as number per mm². The amounts of woven bone and marrow fibrous tissue were determined by point-counting decalcified sections of iliac cancellous bone using the polarizer to identify woven bone.

Parathyroid Glands

These were obtained from 33 patients, usually at necropsy but at operation in three. Glands were individually measured and weighed in most instances. Sections at several levels up to the maximum diameter of each gland were examined histologically after staining with haematoxylin and eosin.
presence of hyperplasia was classified as diffuse (type II) or nodular (type IV) according to Gilmour (1947).

**Classification of Cases**

All patients suffered from azotaemic renal failure with a blood urea of at least 100 mg per 100 ml at the time of study. Many of the biopsy specimens were obtained from selected patients with clinical, biochemical, or radiological evidence of bone disease. The majority of patients coming to necropsy were unselected and represented a more or less consecutive series dying from chronic renal failure in this hospital during the period since 1966. The patients have been classified on the basis of the nature of their renal disorder using histological criteria in 46 patients. Some indication of the duration of azotaemia was obtained by reference to the time interval between the recording of an elevated blood urea (above about 50 mg per 100 ml) and the time of the bone histology. The clinical records were also examined to determine if vitamin D or any other treatment such as steroids or Epanutin had been prescribed and which might have influenced the bone histology.

**Results**

**Parathyroid Glands**

Table I summarizes details of the weights and histology of the parathyroid glands in 33 patients. The glands were hyperplastic in 30 (91%). In the remaining three (cases 19, 23, and 26) there was no convincing evidence of hyperplasia. For example, in case 19, an elderly woman with a long history of azotaemia due to chronic pyelonephritis, the four parathyroid glands were perhaps slightly increased in size but histologically fat cells were still conspicuous and although in some glands there was a predominance of water clear cells there were no mitoses to suggest hyperplasia and the bones did not show histological changes of osteitis fibrosa.

Five of the 30 patients with hyperplastic parathyroid glands (cases 6, 18, 21, 32, and 34) showed severe and nodular hyperplasia of mixed cell type (Gilmour type IV) with the largest and heaviest

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Number of Glands</th>
<th>Parathyroid Gland Weight (mg)</th>
<th>Histology Type(^{1}) (Gilmour, 1947)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>78</td>
<td>94</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>59</td>
<td>76</td>
</tr>
<tr>
<td>6</td>
<td>*3(^{a})</td>
<td>592</td>
<td>713</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>28</td>
<td>63</td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td>37</td>
<td>84</td>
</tr>
<tr>
<td>11</td>
<td>2</td>
<td>39</td>
<td>41</td>
</tr>
<tr>
<td>18</td>
<td>*4</td>
<td>520</td>
<td>570</td>
</tr>
<tr>
<td>19</td>
<td>4</td>
<td>53</td>
<td>80</td>
</tr>
<tr>
<td>20</td>
<td>4</td>
<td>46</td>
<td>72</td>
</tr>
<tr>
<td>21</td>
<td>4</td>
<td>187</td>
<td>89</td>
</tr>
<tr>
<td>22</td>
<td>3</td>
<td>37</td>
<td>62</td>
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<tr>
<td>23</td>
<td>2</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>25</td>
<td>4</td>
<td>30</td>
<td>105</td>
</tr>
<tr>
<td>26</td>
<td>2</td>
<td>62</td>
<td>58</td>
</tr>
<tr>
<td>27</td>
<td>3</td>
<td>126</td>
<td>85</td>
</tr>
<tr>
<td>28</td>
<td>3</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>31</td>
<td>2</td>
<td>78</td>
<td>122</td>
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<tr>
<td>32</td>
<td>4</td>
<td>110</td>
<td>139</td>
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<tr>
<td>34</td>
<td>*4</td>
<td>768</td>
<td>1221</td>
</tr>
<tr>
<td>37</td>
<td>3</td>
<td>34</td>
<td>36</td>
</tr>
<tr>
<td>39</td>
<td>4</td>
<td>28</td>
<td>59</td>
</tr>
<tr>
<td>40</td>
<td>4</td>
<td>49</td>
<td>60</td>
</tr>
<tr>
<td>41</td>
<td>2</td>
<td>87</td>
<td>43</td>
</tr>
<tr>
<td>42</td>
<td>4</td>
<td>47</td>
<td>16</td>
</tr>
<tr>
<td>43</td>
<td>4</td>
<td>43</td>
<td>43</td>
</tr>
<tr>
<td>44</td>
<td>4</td>
<td>28</td>
<td>64</td>
</tr>
<tr>
<td>45</td>
<td>4</td>
<td>30</td>
<td>34</td>
</tr>
<tr>
<td>46</td>
<td>4</td>
<td>71</td>
<td>59</td>
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<tr>
<td>47</td>
<td>4</td>
<td>80</td>
<td>36</td>
</tr>
<tr>
<td>48</td>
<td>4</td>
<td>35</td>
<td>49</td>
</tr>
<tr>
<td>49</td>
<td>4</td>
<td>40</td>
<td>63</td>
</tr>
<tr>
<td>50</td>
<td>4</td>
<td>242</td>
<td>95</td>
</tr>
<tr>
<td>51</td>
<td>3</td>
<td>27</td>
<td>77</td>
</tr>
</tbody>
</table>

Table I Weights and histology of the parathyroid glands in 33 patients with renal disease

\(^{a}\) Surgical specimens.

\(^{1}\) I normal, II diffuse hyperplasia, ID incompletely developed, IV nodular hyperplasia (Gilmour, 1947).
glands observed in this series. The remaining 25 cases showed a diffuse hyperplasia with loss of fat cells and predominance of transitional and/or water clear cells. In five of these the normal structure of the glands was largely replaced (Gilmour type II hyperplasia) whilst in the other 20 some residual fat cells and other features of the normal gland persisted (Gilmour type II hyperplasia, incompletely developed).

**BONE HISTOLOGY**

**Osteitis fibrosa**

Fifty-six of the 60 patients showed some degree of active osteitis fibrosa. The incidence of osteitis fibrosa was similar in the biopsy and necropsy series (table II). However, the severity of osteitis fibrosa differed between the two groups and the severe examples were more frequently observed in the biopsy series (table III). Thus, of the 18 patients with severe or very severe osteitis fibrosa (grade 3 and above), in 13 (72%) bone had been obtained by biopsy. Of the four patients without active osteitis fibrosa (cases 19, 23, 26, and 30), in two (cases 23 and 26) there was a short history of proliferative glomerulonephritis and none showed any other evidence of azotaemic osteodystrophy.

**Woven bone**

The osteoclastic resorption of bone in many patients was accompanied by formation of new woven bone. This was largely mineralized even in patients with osteomalacia and an excess of lamellar osteoid. Fifty-four patients (90%) had some degree of woven bone formation. This was mineralized in 27 cases but in a further 27 although most of the woven bone was mineralized some also appeared as woven osteoid. In examples of severe renal osteodystrophy woven bone accounted for about 50 to 75% of the bone present. Individual values for the amount of woven bone present are given in table V and the mean values with standard errors, expressed as percentages of the measured area and of cancellous bone, in table IV. The values in the two age groups ≤ 50 years and > 50 years are very similar, but in both age groups significantly higher mean values were obtained in the biopsy series in comparison with the necropsy series (table IV). This is attributed to the selected nature of the biopsy specimens.

**Marrow fibrosis**

The amount of marrow fibrous tissue expressed as

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<table>
<thead>
<tr>
<th>Necropsies (37 patients)</th>
<th>Biopsies (25 patients)</th>
<th>Combined (60 patients)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteitis fibrosa</td>
<td>34 (92%)</td>
<td>24 (96%)</td>
</tr>
<tr>
<td>Osteomalacia</td>
<td>10 (27%)</td>
<td>15 (60%)</td>
</tr>
<tr>
<td>Osteosclerosis</td>
<td>7 (19%)</td>
<td>11 (44%)</td>
</tr>
</tbody>
</table>

Table II  Incidence of osteitis fibrosa, osteomalacia, and osteosclerosis

1 In two patients both biopsy and necropsy specimens were available.

---

<table>
<thead>
<tr>
<th>Number of Patients/Observations</th>
<th>Incidence of Osteitis Fibrosa Grades</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Necropsies</td>
<td>37/37</td>
</tr>
<tr>
<td>Biopsies</td>
<td>25/26</td>
</tr>
<tr>
<td>Combined</td>
<td>60/63</td>
</tr>
</tbody>
</table>

Table III  Distribution of severity (grades) of osteitis fibrosa in necropsy and biopsy series

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<table>
<thead>
<tr>
<th>Age Group</th>
<th>Biopsy/ Necropsy</th>
<th>Number of Observations</th>
<th>Woven Bone as Percentage of Measured Area</th>
<th>Cancellous Bone</th>
<th>Marrow Fibrosis as Percentage of Measured Area</th>
<th>Osteoclasts per mm² Cancellous Bone</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 50 yr</td>
<td>Biopsy</td>
<td>22</td>
<td>8-8±1-7 [p &lt;0-025]</td>
<td>25-9±2-1 [p &lt;0-05]</td>
<td>5-6±2-0 [p &lt;0-05]</td>
<td>2-87±0-67 [p &lt;0-01]</td>
</tr>
<tr>
<td></td>
<td>Necropsy</td>
<td>19</td>
<td>3-9±1-0</td>
<td>12-1±2-6 [p &lt;0-05]</td>
<td>0-7±0-1</td>
<td>0-75±0-18 [p &lt;0-05]</td>
</tr>
<tr>
<td></td>
<td>Combined</td>
<td>41</td>
<td>6-5±1-1</td>
<td>19-5±3-2</td>
<td>3-3±1-1</td>
<td>1-89±0-40</td>
</tr>
<tr>
<td>&gt; 50 yr</td>
<td>Biopsy</td>
<td>4</td>
<td>8-3±1-4 [p &lt;0-005]</td>
<td>31-0±5-0 [p &lt;0-02]</td>
<td>4-5±1-5 [p &lt;0-05]</td>
<td>2-23±0-63 [p &lt;0-025]</td>
</tr>
<tr>
<td></td>
<td>Necropsy</td>
<td>18</td>
<td>2-6±0-6 [p &lt;0-005]</td>
<td>13-0±3-1 [p &lt;0-01]</td>
<td>1-5±0-7</td>
<td>0-77±0-25 [p &lt;0-025]</td>
</tr>
<tr>
<td></td>
<td>Combined</td>
<td>22</td>
<td>3-6±0-7</td>
<td>16-3±3-0</td>
<td>2-0±0-7</td>
<td>1-03±0-26</td>
</tr>
<tr>
<td>All cases</td>
<td></td>
<td>63</td>
<td>5-5±0-8</td>
<td>18-4±2-3</td>
<td>2-9±0-8</td>
<td>1-59±0-28</td>
</tr>
</tbody>
</table>

Table IV  Mean values and standard errors for amounts of woven bone and marrow fibrosis and number of osteoclasts in iliac bone

1 Comparisons are between biopsy and necropsy series; p by Student's t test between bracketed values.
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**Case No.** | **Age and Sex** | **Nature of Renal Disease** | **Bone Biopsy (B) Necropsy (N)** | **Grade of Osteitis Fibrosa** | **Waven Bone Percentage of Cancellous Bone** | **Marrow Fibrosis Percentage of Measured Area** | **Number of Osteoclasts per mm² Cancellous Bone**
--- | --- | --- | --- | --- | --- | --- | ---
1 | 31 F | PK | N | 2 | 10.2 | 1-1 | 1-74
2 | 32 M | PK | B | 1-5 | 6.8 | 0-4 | 0-38
3 | 40 M | PK | N | 2 | 7.4 | 0-7 | 0-74
4 | 42 M | PK | B | 4 | 37.2 | 5-4 | 3-47
5 | 45 F | PK | N | 2-5 | 33.6 | 0-6 | 0-67
6 | 45 F | PK | B | 4 | 50.8 | 8-0 | 7-70
7 | 45 F | PK | B | 2 | 12.0 | 0-4 | 0-29
8 | 54 F | PK | N | 2-5 | 19.7 | 1-3 | 0-88
9 | 56 F | PK | N | 3 | 10.0 | 1-0 | 2-37
10 | 58 F | PK | N | 2 | 16.6 | 1-4 | 0-96
11 | 60 F | PK | N | 2 | 8.3 | 0-4 | 0-66
12 | 14 M | CP | B | 2-5 | 22-7 | 3-2 | 2-06
13 | 14 M | CP | B | 5 | 43.7 | 12-1 | 5-12
14 | 35 F | CP | B | 5 | 67-0 | 12-0 | 7-41
15 | 35 F | CP | N | 3 | 36-9 | 1-6 | 1-62
16 | 9 F | CP | B | 4-5 | 53-1 | 9-4 | 6-01
17 | 40 M | CP | B | 2 | 5-3 | 0-7 | 0-96
18 | 49 M | CP | B | 3-5 | 61-6 | 5-7 | 5-00
19 | 55 F | CP | N | 0 | 6-6 | 0 | 0-06
20 | 57 M | CP | N | 2 | 7-6 | 0-9 | 0-71
21a | 60 F | CP | B | 4 | 42-6 | 8-4 | 3-39
21b | 62 F | CP | N | 4-5 | 43-8 | 13-8 | 4-40
22 | 15 M | SG | N | 1-5 | 11-5 | 0-3 | 0-56
23 | 16 F | SG | N | 0 | 5-4 | 0 | 0-02
24 | 37 M | SG | N | 2 | 0 | 0 | 0-30
25 | 52 M | SG | N | 2 | 6-5 | 0-4 | 0-30
26 | 53 M | SG | N | 0 | 0 | 0-1 | 0-01
27 | 23 F | SLE | N | 1 | 9-6 | 0-1 | 0-02
28 | 27 F | CG | N | 2-5 | 23-3 | 2-0 | 2-49
29 | 28 M | CG | B | 1-5 | 0 | 0-3 | 0-42
30 | 35 M | CG | B | 0 | 0 | 0 | 0-01
31 | 42 F | CG | N | 3 | 29-9 | 2-1 | 2-64
32 | 50 M | CG | N | 2-5 | 21-2 | 1-1 | 0-48
33 | 8 M | H, CP | B | 3-5 | 19-1 | 6-9 | 2-30
34 | 30 M | H | B | 5 | 78-5 | 42-4 | 11-10
35a | 32 M | H | B | 1 | 0 | 0 | 0-19
35b | 34 M | H | B | 2 | 8-2 | 0-6 | 0-42
36 | 50 M | AB, CP | B | 2 | 11-1 | 0-1 | 0-19
37 | 70 F | H, CP, M | N | 1 | 0 | 0-1 | 0-11
38 | 31 F | RC, CP | N | 1 | 0 | 0-1 | 0-07
39 | 42 M | RC, CP | N | 2 | 5-1 | 0-4 | 0-79
40 | 51 F | RC, CP | N | 2 | 8-7 | 0-3 | 0-27
41 | 53 M | RCy, CP | N | 2 | 9-6 | 0-7 | 0-36
41 | 54 F | PN, AN | N | 3 | 43-7 | 2-6 | 0-96
43a | 57 F | PN, AN | B | 2-5 | 19-1 | 1-2 | 0-72
43b | 59 F | PN, AN | N | 2 | 23-1 | 1-0 | 0-59
44 | 45 F | HN | N | 2 | 4-3 | 0-3 | 0-36
45 | 50 M | HN | N | 1 | 2-6 | 0-1 | 0-06
46 | 62 M | HN | N | 1 | 2-9 | 0-2 | 0-09
47 | 68 M | HN | N | 2 | 18-3 | 1-6 | 0-35
48 | 43 M | RI | N | 2 | 3-0 | 1-0 | 0-59
49 | 32 M | R. Am. | N | 1 | 0-9 | 0-1 | 0-12
50 | 63 F | R. Am. | N | 1-5 | 6-5 | 0-1 | 0-19
51 | 72 F | R. Am. | N | 2 | 2-4 | 0-6 | 0-52
52 | 28 M | CP | B | 2-5 | 15-7 | 3-8 | 1-85
53 | 42 F | CP | B | 4-5 | 30-7 | 8-7 | 5-60
54 | 42 M | CP | N | 1-5 | 15-5 | 0-8 | 0-46
55 | 43 F | CP | B | 2 | 14-2 | 1-3 | 0-68
56 | 71 F | CP | B | 3-5 | 27-8 | 4-0 | 1-68
57 | 29 M | CG | N | 2 | 10-1 | 0-9 | 0-56
58 | 39 M | CG | B | 3 | 30-0 | 1-8 | 1-89
59 | 39 F | CRF | B | 1 | 1-3 | 0-4 | 0-17
60 | 52 F | CRF | B | 3-5 | 34-5 | 4-5 | 3-13

Table V Details of nature of renal disease and severity of osteitis fibrosa with quantitative histology of woven bone, marrow fibrosis, and number of osteoclasts in 60 patients with renal failure

PK, polycystic kidney; CP, chronic pyelonephritis; SG, subacute glomerulonephritis; SLE, systemic lupus erythematosus; CG, chronic glomerulonephritis; H, hydronephrosis; AB, atonic bladder; M, malignant ureteric stricture; RC, renal calculi; R, Cy, renal cysts; PN, AN, papillary necrosis with analgesic nephropathy; HN, hypertensive nephrosclerosis; RI, renal infarcts; R Am, renal amyloid; CRF², chronic renal failure unknown cause.
Table VI  Results of quantitative histology of mineralized and non-mineralized bone in the ilium in 60 patients with renal failure

1 This biopsy specimen appeared to be obliquely taken and consisted largely of diseased cortical bone. Data from this case excluded from statistical analyses.

m = mineralized bone, pm = partly mineralized bone.
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a percentage of the measured area is given for individual patients in table V. In the severe examples of osteitis fibrosa the values varied from 8.7 to 13.8%. The mean values were similar in both age groups but higher in the biopsy series. This difference between the biopsy and necropsy specimens was only statistically significant in the age group \( < 50 \) years (table IV).

Osteoclasts

Individual values for the number of osteoclasts per mm² of cancellous bone are given in table V. They are significantly in excess of the normal range of 0.01 to 0.03 per mm² and in the more severe examples of osteitis fibrosa varied from 4.4 to 11.1 per mm². The mean values were very similar in both age groups but significantly higher values were again obtained in the biopsy series as a result of selection (table IV).

Hyperosteoedidosis and osteomalacia

There was a statistically significant increase in the amount of osteoid and reduction in the percentage mineralization of bone in the group of patients with renal disease when compared with the control group (tables VI and VII). We have applied the term 'hyperosteoedidosis' or 'excess of osteoid' when the proportion of the measured area comprising osteoid exceeds 0.5%, the upper limit in our 68 control subjects being 0.45% with means of 0.12 ± 0.02% and 0.09 ± 0.02% in the age groups \( < 50 \) years and \( > 50 \) years, respectively. On this basis there were 34 patients (57%) in the series as a whole with hyperosteoedidosis. The incidence was 32 out of 58 patients (55%) when those without excess osteoid but who had received vitamin D are excluded. Twenty-four patients (40%) were considered to have osteomalacia. The incidence of osteomalacia was higher in the biopsy series than in the necropsy series (table II), largely due to the selected nature of the biopsy series. The results of quantitative histology in patients with osteomalacia are summarized in table VIII. Osteomalacia was considered minimal in five patients with only a slight excess of osteoid in the range 1.64 to 3.78% (mean 2.73%) and [89%] mineralization of cancellous bone. However, the excess osteoid could not be explained on a basis of an increase in osteoblastic activity and the calcification front was slightly reduced in extent. In the remaining 19 patients there was mild to severe osteomalacia and in five osteoid accounted for \( > 15 \) % of the measured area. In the mild to severe examples the calcification front was reduced or absent and the maximum number of birefringent lamellae in the osteid seams was increased up to five to 16. The possibility of occult osteomalacia in the other patients with little or no excess of osteoid was excluded by the demonstration of a calcification front at the sites of new bone formation (Bordier and Tun Chot, 1972).

One patient with osteomalacia at the time of biopsy (case 43) was subsequently treated with large doses of vitamin D and at necropsy almost two years later the osteomalacia had resolved and the calcification front was present. The amount of mineralized bone had doubled and the total bone remained unchanged. A further seven patients (cases 2, 8, 11, 21, 36, 41, and 57) were known to have had vitamin D prescribed and this might be the explanation why four of these (cases 2, 11, 41, and 57) did not have osteomalacia. They did have osteitis fibrosa, however. The other two cases (8 and 36) had osteomalacia in spite of vitamin D therapy. Excluding those patients without osteomalacia who had received

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Type of Case</th>
<th>Number of Patients/Observations</th>
<th>Percentage of Measured Area Comprising</th>
<th>Percentage Mineralization of Bone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total Bone</td>
<td>Mineralized Bone</td>
</tr>
<tr>
<td>( &lt; 50 ) yr</td>
<td>All patients</td>
<td>40/41</td>
<td>29.8 ± 1.9 (p &lt; 0.001)</td>
<td>25.9 ± 1.4 (p &lt; 0.001)</td>
</tr>
<tr>
<td></td>
<td>Excluding steroids</td>
<td>36/27</td>
<td>31.0 ± 2.0 (p &lt; 0.001)</td>
<td>26.4 ± 1.5 (p &lt; 0.001)</td>
</tr>
<tr>
<td></td>
<td>Excluding steroids and vitamin D</td>
<td>34/35</td>
<td>31.1 ± 2.1 (p &lt; 0.001)</td>
<td>26.6 ± 1.5 (p &lt; 0.001)</td>
</tr>
<tr>
<td>( &gt; 50 ) yr</td>
<td>All patients</td>
<td>20/21</td>
<td>21.0 ± 1.9 (p &lt; 0.001)</td>
<td>18.1 ± 1.5 (p &lt; 0.001)</td>
</tr>
<tr>
<td></td>
<td>Excluding steroids</td>
<td>18/19</td>
<td>21.1 ± 2.1 (p &lt; 0.001)</td>
<td>17.9 ± 1.7 (p &lt; 0.001)</td>
</tr>
<tr>
<td></td>
<td>Excluding steroids and vitamin D</td>
<td>16/17</td>
<td>21.2 ± 2.1 (p &lt; 0.001)</td>
<td>17.6 ± 1.6 (p &lt; 0.001)</td>
</tr>
</tbody>
</table>

Table VII  Quantitation of mineralized and non-mineralized bone in 60 patients with renal failure

The \( p \) values in brackets indicate the degree of significance of the differences between the respective control and renal group means.
vitamin D the incidence of all grades of severity of osteomalacia was 24 out of 56 (43%). Case 35 showed minimal osteomalacia at the time of the first bone biopsy, was not treated with vitamin D, and at a second biopsy two years later there had been an increase in osteoid from 1·64 to 5·09%.

Ten patients with osteomalacia had areas of partly mineralized bone in the form of linear and irregular granular patches of low mineral density in von Kossa preparations. For brevity only the values for fully and partly mineralized bone combined have been given in table VI and used for statistical comparisons. To illustrate the variation in the amount of partly mineralized bone which may be present, in two patients (cases 13 and 53) the data have been recorded separately in table VI. It can be seen that the amount of partly mineralized bone may be small or almost equal to the amount which is completely mineralized.

Osteosclerosis (figs. 2 and 3)
We have defined osteosclerosis in terms of total bone values observed in our 68 control subjects (Ellis and Peart, 1972) to include patients with total bone values exceeding the control mean plus 3 standard deviations, ie, values > 32·3% for the age group < 50 years and slightly less for elderly subjects (eg, case 56 with total bone 29·3%). On this basis there were 18 patients out of 60 (30%) with osteosclerosis (table II). Osteosclerosis occurred more commonly in patients aged < 50 years. Thus of the 18 examples (93%) were aged < 50 years and only three (17%) were aged > 50 years. This difference was not attributable to a difference in the number of patients in each age group. The incidence of osteosclerosis was higher in the selected biopsy series of patients than it was in the relatively unselected necropsy series (table II). Most patients had at least a little osteoid contributing to the raised total bone and 14 had osteomalacia. The severe examples of osteosclerosis with total bone values > 40·0% were all in patients with osteomalacia. The highest values in patients without osteomalacia and not having received vitamin D were 37·1 and 40·0% in cases 59 and 12, respectively. Two patients (cases 41 and 57) with osteosclerosis and no osteomalacia had been treated with vitamin D. The results of quantitative histology in patients with osteosclerosis are summarized in table IX. All these patients had mineralized woven bone and this was responsible for the high normal or raised mineralized bone value contributing to the osteosclerosis often in the presence of a reduced amount of lamellar mineralized bone. In some patients (cases 47 and 53) with a normal amount of mineralized bone, the total bone was raised as the result of an excess of osteoid. In general,

<table>
<thead>
<tr>
<th>Age and Group</th>
<th>Number of Patients/Observations</th>
<th>Percentage of Measured Area Comprising</th>
<th>Percentage Mineralization of Bone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total Bone</td>
<td>Mineralized Bone</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(p&lt;0·001)</td>
<td>(p&lt;0·001)</td>
</tr>
<tr>
<td>&lt; 50 yr</td>
<td>All cases 17/18</td>
<td>38·1 ± 3·1 (p&lt;0·001)</td>
<td>29·0 ± 2·4 (p&lt;0·001)</td>
</tr>
<tr>
<td></td>
<td>Excluding minimal osteomalacia*</td>
<td>38·4 ± 3·7 (p&lt;0·001)</td>
<td>28·1 ± 2·8 (p&lt;0·005)</td>
</tr>
<tr>
<td>&gt; 50 yr</td>
<td>All cases 7/8</td>
<td>25·6 ± 3·5 (p&lt;0·001)</td>
<td>19·2 ± 2·7 (p&lt;0·10)</td>
</tr>
<tr>
<td></td>
<td>Excluding minimal osteomalacia*</td>
<td>24·7 ± 4·7 (p&lt;0·001)</td>
<td>17·1 ± 3·2 (p&lt;0·10)</td>
</tr>
</tbody>
</table>

Table VIII Quantitation of mineralized and non-mineralized bone in patients with osteomalacia

1Minimal osteomalacia defined as > 89% mineralization of cancellous bone. P values in brackets indicate degree of significance of differences between mean values for control and renal groups.

<table>
<thead>
<tr>
<th>Group and Number</th>
<th>Percentage Measured Area Comprising</th>
<th>Percentage Mineralization of Cancellous Bone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Bone</td>
<td>Mineralized Bone</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(p&lt;0·001)</td>
</tr>
<tr>
<td>All cases of osteosclerosis (18)</td>
<td>41·6 ± 2·4 (p&lt;0·001)</td>
<td>33·1 ± 1·8 (p&lt;0·001)</td>
</tr>
<tr>
<td>Cases of osteosclerosis excluding those receiving vitamin D (14)</td>
<td>41·2 ± 2·5 (p&lt;0·001)</td>
<td>32·4 ± 2·1 (p&lt;0·001)</td>
</tr>
</tbody>
</table>

Table IX Quantitation of mineralized and non-mineralized bone in 18 patients with osteosclerosis

1P values in brackets indicate degree of significance of differences between means for control and renal groups.
Azotaemic renal osteodystrophy: a quantitative study on iliac bone

Fig. 2 Section of iliac bone showing diffuse osteosclerosis in case 54. The fissures are artefact. Undecalcified. v. Kossa × 6·6

Fig. 3 Osteosclerosis. Part of fig 2 showing excess mineralized bone and osteoid covering most of the bone surface. Undecalcified. v. Kossa × 25
although some woven bone appeared as osteoid, the bulk of the osteoid present in osteosclerosis was lamellar in type comprising broad seams with up to 16 birefringent lamellae covering almost the entire bone surface.

Osteoporosis
In many patients the total bone value was within the normal range but such an isolated value in any individual is to some extent meaningless unless considered with the histological changes, since similar values may occur in bones with widely differing proportions of mineralized lamellar or woven bone and osteoid. Taking into account the effect of age, the total bone value and the histological appearances there were seven patients with reduced total bone (cases 1, 11, 21, 40, 44, 49, and 50). The low total bone value in case 49 could probably in part be attributed to the administration of prednisone in the previous 19 months. A further two patients (cases 22 and 27) with values near the lower limit of normal (16·4 and 17·8%, respectively) had also received steroids. In the remaining five patients treated with steroids (cases 23, 25, 26, 29, and 35) the total bone values were well within the normal range. Excluding patients treated with steroids, osteoporosis was not a prominent feature in the present series and occurred in only five cases (8%). A further case (case 21) had osteoporosis and osteomalacia.

Other changes in mineralized bone
Mineralized bone was elevated in 11 patients (18%) due to the formation of woven bone, within the normal range in 37 patients (62%), and reduced in 12 (20%). Seven of those with low values also had low total bone, the one (case 49) having been treated by steroids. The figure obtained for mineralized bone by point counting of undecalcified sections represents the sum of the amounts of mineralized woven and lamellar bone present, and, although we do not have data on the amounts of lamellar mineralized bone, this is clearly reduced in many cases, especially in those with severe degrees of hyperparathyroidism.

Infractions of bone
In one patient (case 21) with severe osteitis fibrosa and osteomalacia there were multiple areas of recent infraction in one iliac crest at necropsy. In a further patient (case 13) with severe osteitis fibrosa and severe osteomalacia transiliial bone biopsy included an area of cortical bone infraction with formation of subperiosteal and endosteal bone

Fig. 4 Infraction showing mineralized (black) remains of lamellar cortical bone with osteoid at the surface and the newly-formed, partly mineralized woven bone in case 13. Undecalcified. v. Kössa × 65
Azotaemic renal osteodystrophy: a quantitative study on iliac bone

callus. This consisted largely of trabeculae of woven bone with intervening fibrous marrow, and some trabeculae showed central mineralization in spite of the fact that the lamellar bone at the surface of neighbouring cancellous trabeculae was not mineralized (fig. 4).

CORRELATIONS

Hyperparathyroidism and nature of renal disease
The five patients with the heaviest glands showing nodular (Gilmour type IV) hyperplasia suffered from a variety of renal diseases as did the patients with more modest sized glands and diffuse (Gilmour type II) hyperplasia. Patients with renal vascular disease had glands of normal weight with mild hyperplasia histologically. The more severe degrees of osteitis fibrosa occurred in patients with polycystic kidneys, chronic pyelonephritis, and obstructive nephropathy. All the patients with grade 4-5 to 5 osteitis fibrosa had chronic pyelonephritis with, in one individual, an obstructive nephropathy.

Hyperparathyroidism and duration of azotaemia
Attempts to relate the parathyroid gland weights and the severity of osteitis fibrosa to the duration of azotaemia were unsuccessful, since the known period of azotaemia probably only occasionally approximates to the actual period of azotaemia. The five patients with nodular parathyroid gland hyperplasia had been azotaemic for at least four to eight years. On the other hand, there were four patients (cases 5, 10, 11, and 19) who had been azotaemic for minimum periods of four to seven years with only slightly enlarged glands and diffuse hyperplasia. The highest mean gland weight observed up to the end of the third year of azotaemia was 73 mg in case 3 and larger glands only occurred after azotaemia of at least four to five years' duration. There was a wide scatter of osteitis fibrosa grades in relation to the known period of azotaemia and in general no useful conclusions can be drawn. Case 19 was exceptional in that this was the only individual with an osteitis fibrosa grade zero, and no definite parathyroid gland hyperplasia after a prolonged period of azotaemia (6 yr 8 mth). In contrast, there was histological evidence of early parathyroid hyperplasia and corresponding changes of osteitis fibrosa in cases 22 and 25 with subacute glomerulonephritis and known periods of azotaemia of only seven and 17 months respectively.

Parathyroid hyperplasia and osteitis fibrosa
The relationship between the mean parathyroid gland weight and the severity (grade) of osteitis fibrosa was determined in 30 patients. Cases 49 and 50 were excluded since amyloid infiltration of the glands gave misleadingly high weights and in case 23 no weights were available. In general the largest glands were associated with the severe grades of osteitis fibrosa (grades 3-5 to 5). Glands of normal weight or only slightly heavier than normal were associated with lesser degrees of osteitis fibrosa. There were occasional exceptions, eg, case 42 with normal sized glands, incomplete type II hyperplasia, and grade 3 osteitis fibrosa, and case 32 with heavy glands and type IV hyperplasia with only grade 2-5 osteitis fibrosa.

The relationships between mean parathyroid gland weights and quantitated features of osteitis fibrosa such as the number of osteoclasts and amount of marrow fibrosis in cancellous bone are given in table X. There was a significant correlation in each instance although there was some initial increase in the number of osteoclasts and the amount of marrow fibrosis without significant change in the parathyroid gland weights.

<table>
<thead>
<tr>
<th>Correlation between</th>
<th>Correlation Coefficient (r)</th>
<th>Degrees of Freedom</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parathyroid weight (mg) : Number osteoclasts (per mm²)</td>
<td>0.928</td>
<td>28</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Parathyroid weight (mg) : Marrow fibrosis (% measured area)</td>
<td>0.903</td>
<td>28</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Parathyroid weight (mg) : Woven bone fibrosis (% measured area)</td>
<td>0.711</td>
<td>28</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Parathyroid weight (mg) : Woven bone fibrosis (% of cancellous bone)</td>
<td>0.822</td>
<td>28</td>
<td>P &lt; 0.001</td>
</tr>
</tbody>
</table>

Table X Correlations between mean parathyroid gland weights and numbers of osteoclasts, amounts of woven bone, and marrow fibrosis

<table>
<thead>
<tr>
<th>Correlation between</th>
<th>Degrees of Freedom</th>
<th>Correlation Coefficient (r)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteoclasts (per mm²) : Marrow fibrosis (% measured area)</td>
<td>60</td>
<td>0.895</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Osteoclasts (per mm²) : Woven bone fibrosis (% of cancellous bone)</td>
<td>50</td>
<td>0.852</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Osteoclasts (per mm²) : Woven bone fibrosis (% of cancellous bone)</td>
<td>60</td>
<td>0.684</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Woven bone fibrosis (% measured area) : Marrow fibrosis (% measured area)</td>
<td>60</td>
<td>0.689</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Table XI Interrelationship between number of osteoclasts and amounts of woven bone and marrow fibrosis
Hyperosteoidosis

There was a positive correlation between the severity of osteitis fibrosa and the amount of woven bone present, and between the parathyroid gland mean weights and amounts of woven bone expressed as a percentage of cancellous bone or as a percentage of the measured area (table X). The large nodular hyperplastic parathyroid glands were associated with much woven bone formation and marrow fibrosis. There were significant correlations between the number of osteoclasts and amounts of marrow fibrosis and woven bone (table XI).

Hyperparathyroidism and amounts of total bone, mineralized bone, and osteoid

There was no precise relationship between the weight of parathyroid glands or severity of osteitis fibrosa and the amounts of total bone, mineralized bone, or osteoid. The severity of osteitis fibrosa varied from grade 1 to grade 5 in the 18 patients with osteosclerosis. There was also a variable relationship between the severity of hyperparathyroidism and the amount of osteoid present. In some instances, e.g., cases 4, 16, 18, and 34, there was severe osteitis fibrosa (grades 3-5 to 5) and very little excess osteoid; in others, e.g., cases 13 and 53, there was severe osteitis fibrosa (grades 5 and 4-5) and osteomalacia; and in others, e.g., cases 47 and 54, there was modest osteitis fibrosa (grades 2 and 1-5) with severe osteomalacia. The four patients with severe osteitis fibrosa and little osteoid had not had vitamin D prescribed. Four of the five patients with nodular hyperplasia of the parathyroid glands and severe osteitis fibrosa (grades 3-5 to 5) had only a modest excess of osteoid with osteomalacia in two individuals.

Hyperosteoideosis and nature and duration of renal disease

Hyperosteoideosis with or without osteomalacia occurred in each of the main groups of renal disease except where there was a short history, for example, in those patients with subacute glomerulonephritis and in one (case 37) with malignant ureteric obstruction and hydronephrosis. Osteomalacia was observed in approximately the same proportion of individuals with polycystic kidneys (55%), chronic pyelonephritis (56%), and chronic glomerulonephritis (43%), but in only one patient (20%) of those with renal vascular disease. The four patients (cases 13, 36, 53, and 54) with osteoid values above 15-0% all had chronic pyelonephritis with or without an obstructive uropathy. One of these was a child (case 13).

Osteosclerosis and nature of renal disease

Osteosclerosis was observed in association with a variety of renal diseases. Amongst the group of 11 patients with both raised mineralized bone and excess osteoid eight had chronic pyelonephritis (alone or in combination with an obstructive lesion) and two had chronic glomerulonephritis. In the remaining case the nature of the renal disease was uncertain. Of the seven patients with a normal amount of mineralized bone and excess osteoid causing osteosclerosis, four had chronic pyelonephritis, two chronic glomerulonephritis, and one hypertensive nephrosclerosis.

Bone symptoms, nature of renal disease, and bone changes

The three children with bone symptoms (cases 12, 13, and 33) had chronic pyelonephritis in association with hydronephrosis in case 33. Two had osteomalacia (cases 13 and 33) and all three had moderate to severe osteitis fibrosa (grades 2-5, 5, and 3-5, respectively).

Of the 12 adults with bone symptoms three had polycystic kidneys (cases 6, 7, and 8), six chronic pyelonephritis (cases 14, 18, 21, 52, 54, and 56), one hydronephrosis (case 34), and one analgesic nephropathy (case 43). The nature of the renal disease in the remaining patient (case 60) was unknown. One patient (case 52) had previously suffered from bone disease as a child. Ten of the adults had osteomalacia (83%), an incidence about double that in the series as a whole. All had some degree of osteitis fibrosa but this was no more severe in some patients with bone symptoms than in those without. However, in five patients (cases 6, 14, 18, 34, and 60) hyperparathyroidism appeared to be the predominant factor and in three of these, cases 6, 18, and 34 with osteitis fibrosa grades 4, 3-5, and 5 respectively, very large nodularly hyperplastic parathyroid glands were removed surgically. The high incidence of bone symptoms is due to the fact that in 10 of the 12 adults affected bone biopsy had been carried out selectively because of symptoms with biochemical and/or radiological evidence of bone disease. In the relatively unselected necropsy series only three out of 35 (cases 8, 21, and 43) had bone symptoms.

Discussion

We have previously (Ellis and Peart, 1972) discussed the variations which may occur when quantitating mineralized and non-mineralized bone in different parts of the iliac bone. The amounts of total bone and osteoid in our cases of chronic renal failure are so consistently different from the amounts observed in our control material as to be significant. In the age group < 50 years this is also true for mineralized bone. Furthermore the values are abnormal in
biopsy and necropsy material where the site selection is under greater control. However, when serial biopsies are studied in the individual patient too much reliance should not be placed upon minor changes in the amounts of mineralized or non-mineralized bone, particularly when a small-bore trephine has been used. In view of the patchy distribution of osteitis fibrosa between and even within individual bones one must accept that in some instances, particularly if an iliac crest biopsy is small, it is possible to overlook minor degrees of osteitis fibrosa. In the present series this was not a problem since multiple bones were available for study at necropsy in three of the four patients without osteitis fibrosa, and a negative grading was on the basis of an iliac bone biopsy in only one individual. We have encountered all grades of severity of azotaemic renal osteodystrophy amongst our cases with varying combinations of osteitis fibrosa, osteomalacia, and osteosclerosis and will discuss the main features in turn.

**Parathyroid Glands**

It is well established that there is secondary parathyroid hyperplasia in chronic renal failure (Pappenheimer and Wilens, 1935; Gilmour and Martin, 1937; Castleman and Mallory, 1937; Herbert, Miller, and Richardson, 1941; Gilmour, 1947; Roth and Marshall, 1969), and we have now demonstrated a significant correlation between the weights of the parathyroid glands, the number of osteoclasts, and amounts of marrow fibrosis and woven bone in cancellous bone. Our findings are consistent with the generally accepted view that in most instances the glands are moderately and diffusely hyperplastic and may still be within the normal weight range or only slightly increased in weight. In a few patients there is a more marked and nodular hyperplasia of the glands with severe osteitis fibrosa giving rise to clinical symptoms and radiological changes corresponding to those of primary hyperparathyroidism.

Although in most instances there is little difficulty in the histological diagnosis of parathyroid hyperplasia problems may arise when there are early changes and the glands are of normal size and weight and to some extent the assessment may then be subjective. The presence of a compact structure with loss of fat cells is a useful guide but caution is needed in the case of the glands of children and young adults who may normally have few or no fat cells present (Ellis and Knight, 1969). Like others (Gilmour, 1947; Christie, 1967) we have observed increased numbers of oxyphil cells in the parathyroid glands of some patients with chronic renal failure particularly those with Gilmour type IV nodular hyperplasia where aggregates of these cells form nodules easily visible to the naked eye. Amyloid infiltration of the parathyroid glands was extensive in two of our three patients with generalized amyloidosis and made a significant contribution to the gland weight. It was less conspicuous in the other case. It differed in distribution from the intracortical amyloid deposits which have been described in about 10% of cases of primary hyperparathyroidism (Leedham and Pollock, 1970).

**Osteitis Fibrosa**

It is difficult to quantitate histologically the precise severity of osteitis fibrosa, since the changes may be patchily distributed in compact and cancellous bone. It is possible to determine the proportion of bone surfaces showing resorption either in sections or microangiographs (Sedlin, Villanueva, and Frost, 1963; Byers and Smith, 1971) but in azotaemic osteodystrophy, especially after haemodialysis, factors other than hyperparathyroidism might be responsible for resorption or resorption fronts may become covered by osteoid which resists resorption. The present system of grading is only semi-quantitative but has the advantage of taking into account changes in both compact and cancellous bone. Biopsy samples of ilium contain variable proportions of compact and cancellous bone and our quantitative observations on the number of osteoclasts and amount of marrow fibrosis have therefore been made on cancellous bone. Further problems are that the cortical bone may be more severely affected and/or cortical bone resorption and new bone formation may make it difficult to distinguish between cancellous and compact bone. We have found the grading system reproducible when re-examining bone sections at intervals of many months and it is a useful means of conveying information concerning the severity of osteitis fibrosa in clinical reports. The system does not lend itself to the usual statistical analyses since it is only semi-quantitative and non-parametric. This is illustrated by the fact that although there is a definite relationship between the osteitis fibrosa grades and number of osteoclasts or amount of marrow fibrosis this is not a linear relationship and there is overlap of individual values between adjacent grades (table XII).

Although osteitis fibrosa was present in most patients (93%), those with severe grades of 4.5 to 5 suffered from chronic pyelonephritis or obstructive nephropathy. The incidence of osteitis fibrosa was similar in patients studied by bone biopsy or at necropsy. The fact that most of the severe examples of osteitis fibrosa (grades 3-5 to 5) were found in the biopsy series is a reflection of case selection since these patients often had severe clinical or radiological...
changes of bone disease.

It is not surprising that there is no precise relationship between the severity of osteitis fibrosa and the amounts of total bone, mineralized bone, or osteoid in the present series. Thus in hyperparathyroidism resorption of mineralized lamellar and woven bone tends to reduce the total and mineralized bone but this is offset to a variable degree by the formation of new woven bone which is largely mineralized. In patients with accompanying osteomalacia an excess of osteoid, mainly lamellar in type, also makes a significant contribution to the total bone mass.

It is commonly accepted that osteoclasts are responsible for bone resorption but in recent years attention has been increasingly focused on the part played by osteocytes (Bélanger, 1965; Taylor and Bélanger, 1969). This process of osteolysis has been described in patients with metabolic bone disease including hyperparathyroidism (Riggs, Kelly, Jowsey, and Keating, 1965) in microradiographs of bone sections. We have not been impressed with the occurrence of bone resorption in the vicinity of osteocytes in our present histological material although we have observed the phenomenon in some early cases. With the accumulation of woven bone there are commonly to be observed large osteocytic lacunae but this is a normal feature of such bone and does not indicate osteolysis.

**Woven bone**

Woven bone formation was a common occurrence in our cases and was present in 90% of patients. The woven bone appeared to be gradually replacing the original mineralized lamellar bone as this was resorbed by osteoclasts but it too was subject to resorption. In some instances 50 to 75% of the bone was in the form of woven bone and some trabeculae consisted entirely of this type of bone. We have been able to demonstrate a significant correlation between the amount of woven bone present and other features of osteitis fibrosa, namely, the number of osteoclasts and amount of marrow fibrosis. The mean weight of parathyroid glands was also related to the amount of woven bone present and to the number of osteoclasts and amount of marrow fibrosis. We have therefore come to regard woven bone formation as an integral part of the repair reaction in hyperparathyroidism. It is of interest to note here that we have subsequently obtained repeat iliac bone biopsies from cases 6, 18, and 34 at periods of 39, 14, and 17 months respectively, after parathyroidectomy. All specimens showed a striking reduction in osteoclastic activity and marrow fibrosis but woven bone persisted. It seems that in renal failure woven bone is only slowly resorbed and replaced by lamellar bone after parathyroidectomy whereas the other changes of osteitis fibrosa resolve relatively early. In relation to this new bone formation in the hyperparathyroidism of renal azotaemia it is of interest to note that Kalu, Pennock, Doyle, and Foster (1970) found increased amounts of mineralized bone in the femora of rats treated with parathormone, apparently the result of accelerated bone formation.

The dimension and contour of the mineralized component of the cancellous trabeculae represent the result of the existing balance between the amount of resorption of lamellar and woven mineralized bone and the amount of newly formed mineralized woven bone. Aggradation of mineralized woven bone commonly offsets the degradation of mineralized bone brought about by osteoclasts and sometimes results in an excess of mineralized bone which contributes to the occurrence of osteosclerosis. In 15 patients (25%) the amount of mineralized lamellar and woven bone together was at the upper limit of the normal range or exceeded it. Gilmour (1947) also reported this replacement of the original lamellar bone by woven bone in patients with renal disease and osteitis fibrosa. Garner and Ball (1966) noted an increased amount of mineralized bone in two non-osteomalacic and four osteomalacic patients in their selected series of 18 cases, an incidence of 33%. They suggested that woven bone might be

<table>
<thead>
<tr>
<th>Osteitis Fibrosa Grade</th>
<th>0</th>
<th>1</th>
<th>1.5-2</th>
<th>2.5-3</th>
<th>3.5-4</th>
<th>4.5-5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of observations</td>
<td>4</td>
<td>8</td>
<td>26</td>
<td>12</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Number of osteoclasts per mm²</td>
<td>0.03 ± 0.01</td>
<td>0.10 ± 0.02</td>
<td>0.55 ± 0.06</td>
<td>1.55 ± 0.22</td>
<td>3.81 ± 0.76</td>
<td>5.71 ± 0.50</td>
</tr>
<tr>
<td>Amount of marrow fibrous tissue as % of measured area</td>
<td>0.02 ± 0.02</td>
<td>0.15 ± 0.04</td>
<td>0.65 ± 0.08</td>
<td>1.86 ± 0.27</td>
<td>6.13 ± 0.64</td>
<td>11.20 ± 0.94</td>
</tr>
</tbody>
</table>

Table XII **Relationship between number of osteoclasts and amount of marrow fibrosis in cancellous bone and osteitis fibrosa gradings**

1Values are means ± 1 standard error.

2Sixty-two observations in 59 patients, case 34 omitted.
contributing to this excess of mineralized bone and subsequently reported woven bone in 14 of 16 cases of azotaemic osteodystrophy (Ball and Garner, 1966). They pointed out that the formation of woven bone had previously been regarded as a late manifestation of azotaemic osteodystrophy (Ball, 1960; Stanbury, Lumb, and Nicholson, 1960), whereas now it was apparent that mineralized woven bone may occur in areas of severe osteitis fibrosa in the malacic phase of renal osteodystrophy. We now wish to extend this to include the pre-malacic phase of renal osteodystrophy in many individuals and to stress that osteitis fibrosa with resorption of mineralized bone commonly precedes the development of osteomalacia in renal osteodystrophy with or without osteosclerosis. In case 35 where there was little woven bone formation the mineralized bone diminished over a two-year period between biopsies whilst the amount of osteoid increased.

Ball and Garner (1966) have described how woven bone may be mineralized in fracture callus or osteitis fibrosa in the presence of osteomalacia. This has been our experience too and in about half our patients with woven bone this was mineralized whilst in the remainder most woven bone was mineralized but some woven osteoid also occurred. Occasionally this woven osteoid was sufficient to make a significant contribution to the absolute amount of osteoid present. The mineralization of woven bone in patients with osteomalacia stresses the importance of the nature of the substrate in bone mineralization processes.

**OSTEOMALACIA**

There is some difficulty in defining osteomalacia in terms of quantitative histology alone (Garner and Ball, 1966; Ellis and Peart, 1972) since excess of osteoid may merely be the result of osteoblastic activity over a greater proportion of bone surfaces than is normal, the seams remain normal in width, and there is no mineralization defect. Some authors have quantitated the proportion of bone surfaces covered by osteoid but this is generally non contributory in renal osteodystrophy since osteomalacia may exist when active formation of osteoid is very patchily distributed at sites of previous resorption but individual seams are abnormally wide, and, on the other hand, be absent when almost all surfaces are covered by active seams of normal width. In many instances of osteomalacia virtually all cancellous bone surfaces are covered by wide osteoid seams. Small amounts of non-mineralized woven bone may also contribute to the osteoid value obtained, and whilst this is relatively unimportant in obvious cases of osteomalacia with a great excess of lamellar osteoid, it may account for a more significant proportion of the total osteoid in borderline cases. Another source of difficulty is that a mineralization defect due to lack of vitamin D can apparently exist in the absence of osteomalacia in the true histological sense, ie, the osteoid seams may be of near normal distribution and width. The only satisfactory histological means of diagnosing osteomalacia takes account of the amount of osteoid, the degree of osteoblastic activity, and the presence and distribution of the calcification front, the latter being reduced or absent in osteomalacia (Bordier and Tun Chot, 1972). It is for these reasons that we have accepted as osteomalacic only those cases showing an excess of the absolute amount of osteoid, usually increased width of lamellar seams as judged by the presence of five or more birefringent lamellae and a reduced or absent calcification front. On this basis and discounting those in whom vitamin D may have been responsible for the lack of excess osteoid, 55% of our patients had an excess of osteoid and 43% osteomalacia. It will be noted that both these figures are considerably less than that for the incidence of osteitis fibrosa (93%). As mentioned previously (Ellis and Peart, 1972), we have encountered difficulty in the application of the solochrome cyanine R technique to demonstrate the calcification front (Matrajt and Hioco, 1966) in low viscosity nitrocellulose-embedded material. It is for this reason that we have come to rely upon the demonstration of the front in undecalciﬁed sections stained by toluidine blue or haematoxylin and by the lipid staining technique of Irving (1958).

The covering of cancellous trabeculae by osteoid renders their surfaces smooth in spite of previous irregular resorption of the underlying mineralized bone. The osteoid seams are apparently resistant to resorption by osteoclasts but resorption of mineralized lamellar bone and woven bone continues deep to the osteoid. With tunnelling by osteoclasts the mineralized bone may be eroded beneath the osteoid and part of the seam is left, with marrow elements on each side. Sometimes a thin strip of mineralized bone remains on the underside of the osteoid seam and when further osteoid is formed deep to this a strip of mineralized bone is embedded in osteoid. This may give rise to a misleading appearance which suggests that the laying down of osteoid has been temporarily interrupted by the formation of a few lamellae of mineralized bone. In some cases of osteomalacia in Von Kóssa preparations part of the osteoid appears lightly stippled indicating some attempt at mineralization and this too is occasionally in a band-like arrangement (fig. 5).

Although all the patients with the highest osteoid values had chronic pyelonephritis, osteomalacia occurred in patients with polycystic kidneys and
chronic glomerulonephritis in comparable numbers. The incidence of excess osteoid and osteomalacia in the present series is lower than we expected since it has been generally considered that osteomalacia is a common bony abnormality in renal osteodystrophy. However, too much reliance cannot be placed upon the existing literature in this respect since some papers have been mainly concerned with the parathyroid glands and osteitis fibrosa and undecalcified sections have not been examined consistently or have the cases been selected as severe examples of renal osteodystrophy. Our findings do not support the view that osteomalacia is possibly the most frequent abnormality in azotaemic osteodystrophy or that vitamin D resistance must be the first and most fundamental abnormality leading to increased parathyroid secretion (Ball, 1960; Stanbury, 1968; Stanbury, Lumb, and Mawer, 1969), but favour the idea that osteomalacia is often preceded by osteitis fibrosa. Thus many of our patients had osteitis fibrosa without an excess of osteoid and histological evidence of parathyroid hyperplasia and osteitis fibrosa was observed in the few patients with relatively short-lived azotaemia as a result of subacute proliferative glomerulonephritis. Our findings support the view that secondary hyperparathyroidism is the commonest histological manifestation of renal osteodystrophy and may often be the first in a series of changes to develop.

It is of interest that in one of our patients given vitamin D a moderately severe degree of osteomalacia underwent complete healing during a period of almost two years with reappearance of a calcification front. Two of our patients, however, still had osteomalacia in spite of vitamin D therapy. The patient who responded to vitamin D therapy showed little if any change in the severity of osteitis fibrosa histologically. In contrast others (Stanbury, 1968) have observed that when the defective mineralization is healed in azotaemic renal osteodystrophy by appropriate doses of vitamin D the secondary hyperparathyroidism is also reversed, at least as judged radiologically.

Histological descriptions of infractions or Looser's zones in osteomalacia are singularly rare (Ball, 1960; Ball and Garner, 1966). Transilial biopsy from one of our patients included part of what we take to be a Looser's zone. This showed subperiosteal and endosteal new bone formation which was largely woven in type. The trabeculae of this woven bone were mineralized to varying degrees although the lamellar bone covering adjacent original cancellous trabeculae was not mineralized. It seems probable that Looser's zones are areas of low radiodensity due to infraction with formation of callus which is largely patchily mineralized woven bone and partly non-mineralized lamellar bone.

**OSTEOSCLEROSIS**

Osteosclerosis has been increasingly recognized as a feature of renal osteodystrophy in recent years (Ginzler and Jaffe, 1941; Gilmour, 1947; Claireaux, 1953; Crawford, Dent, Lucas, Martin, and Nassim, 1954; Kaye, Pritchard, Halpenny, and Light, 1960; Haust et al., 1964). Most of these descriptions were based on selected material and the criteria varied. Garner and Ball (1966), applying more critical quantitative histological techniques, found an increase in the iliac crest total bone values in 13 out of 18 selected cases of azotaemic osteodystrophy. In our partly selected and partly unselected series of 60 patients we considered that there was osteosclerosis in 18, ie, 30% of cases. The incidence of osteosclerosis in our relatively unselected series of necropsied patients was only seven out of 37 (19%). Our severe examples of osteosclerosis with total bone values exceeding 40% occurred in patients with osteomalacia and 11 of the 13 patients of Garner and Ball (1966) with osteosclerosis also had osteomalacia. We agree with them that osteosclerosis, as defined in terms of a raised total bone mass histologically, is
Azotaemic renal osteodystrophy: a quantitative study on iliac bone

commonly due to an accumulation of non-mineralized bone and that mineralized bone is often also increased in these patients. Thus in the present series, of the 18 patients with osteosclerosis the mineralized bone values were at the upper range of normal in four and raised above normal in 11. Ball and Garner (1966) suggested that the increased mineralized bone was attributable to lack of resorption of lamellar mineralized bone and formation of new woven mineralized bone. Although we do not agree that there is lack of resorption of mineralized bone in these patients our results amply confirm that woven bone accounts for the excess of mineralized bone which may be present. It is clear that an increase in mineralized bone may occur in spite of excessive resorption of lamellar mineralized bone and even in the presence of osteomalacia provided there is sufficient new mineralized woven bone formed. Occasionally it would appear that osteosclerosis can arise as the result solely of an excess of lamellar mineralized bone, but we have not encountered this type of case.

osteoporosis

Osteoporosis was not a common feature of the bone changes in the present series. Although it might be thought that the excessive resorption of bone associated with hyperparathyroidism would lead to a reduction in total bone this is not usually the case since the loss is compensated for by the formation of woven bone. In the majority of instances the amount of mineralized lamellar bone is actually reduced, and, although quantitatively the amount of mineralized bone present appears normal, it is qualitatively abnormal.

Pathogenesis of Azotaemic Renal Osteodystrophy

It is evident that azotaemic renal osteodystrophy if sought histologically is extremely common in patients with chronic renal failure due to a wide variety of renal disorders. Osteitis fibrosa due to secondary hyperparathyroidism is the most frequent finding. There is variable degradation of the original mineralized lamellar bone and aggradation of largely mineralized woven bone with accompanying marrow fibrosis. The balance of these gradational processes is usually such that the amount of mineralized bone remains within normal limits or may be elevated. Osteomalacia occurs less commonly but the excess osteoid then contributes significantly to the total bone mass sometimes causing osteosclerosis. We believe that the absence of a significant reduction in mineralized bone in renal osteodystrophy is usually not explicable on the basis of an absence of a pre-alacic resorption of bone (Garner and Ball, 1966) but occurs in spite of this resorption due to the formation of woven mineralized bone.

In view of the differing incidences of osteitis fibrosa and osteomalacia it appears that osteomalacia is not the earliest or commonest bone abnormality in chronic renal failure as was at one time suggested (Stanbury, 1968), but that osteitis fibrosa is usually followed by the development of osteomalacia. Bricker, Ogden, Schreiner, and Walser (1969) have emphasized that secondary hyperparathyroidism may occur early in renal failure as a response to hypocalcaemia and hyperphosphataemia and this view is fully consistent with our histopathological observations. There is also accumulating supporting evidence from serum parathormone studies (Bernson and Yalow, 1966; Reiss, Canterbury, and Kanter, 1969; O'Riordan, Page, Kerr, Walls, Moorhead, Crockett, Franz, and Ritz, 1970; Fournier, Arnaud, Johnson, Taylor, and Goldsmith, 1971). It was held (Stanbury and Lumb, 1962, 1966) that the initial stimulus to secondary hyperparathyroidism was hypocalcaemia resulting from the effects on bone of acquired vitamin D resistance and that osteitis fibrosa occurred only in patients with advanced renal failure. In the light of recent knowledge derived from parathormone studies Stanbury (1972) now concedes that this concept of azotaemic renal osteodystrophy is incorrect.

It appears then that vitamin D resistance is not a phenomenon of early renal failure. Although its time of onset may be related to the level of body stores of vitamin D at the outset of progressive renal failure (Lumb, Mawer, and Stanbury, 1971), hyperparathyroidism itself may induce osteomalacia. Recent studies have revealed several metabolites of vitamin D and that the kidney is the sole site of formation of 1,25-dihydroxycholecalciferol (1,25-DHCC) from 25-hydroxycholecalciferol (25-HCC) (Kodicek, 1972). It has also been shown (Galante, MacAuley, Colston, and MacIntyre, 1972) that parathormone may interfere with production by the kidney of this active factor 1,25-DHCC. This inhibition of the conversion of 25-HCC to 1,25-DHCC by parathormone may partly explain the development of osteomalacia in the secondary hyperparathyroidism of renal failure. Progressive destruction of renal tissue in chronic renal disease might also be expected to interfere with and finally prevent the formation of 1,25-DHCC causing increasingly severe osteomalacia. In keeping with this concept Stanbury (1972) refers to unpublished work in which he and his colleagues have found that the formation of 1,25-DHCC is reduced in patients with chronic renal failure and in most instances there is no formation at all.

Mention should also be made of the possible effects
of fluoride retention in the pathogenesis of osteomalacia and osteosclerosis in renal failure. Chronic renal failure is known to cause progressive retention of fluoride and increased amounts of fluoride have been demonstrated in bone in cases of renal osteosclerosis (Kaye et al, 1960). This may be of particular importance in patients treated by intermittent haemodialysis and in whom the serum fluoride levels may be elevated (Siddiqui, Simpson, Ellis, and Kerr, 1970).

In conclusion, our results indicate that in any series of patients commencing intermittent haemodialysis there will be variations between individual patients regarding the precise nature and severity of the bone changes. The majority may be expected to show some degree of osteitis fibrosa and a variable proportion would also have osteomalacia with or without osteosclerosis depending upon the nature of the underlying renal disease, in particular as this relates to the duration of azotaemia before haemodialysis becomes a necessity. One must be fully cognizant of the variety of the bone changes in patients commencing haemodialysis in order to assess the further bone changes found after differing periods of dialysis.

We wish to acknowledge the help of many of our colleagues in the Departments of Medicine and Pathology, and in particular we are indebted to Professor D. N. S. Kerr, Dr J. Walls, Dr J. Siddiqui, and Dr A. R. Morley. We also wish to thank Dr J. Ball for his help and advice at the outset of this study and Messrs J. R. Maclennan and J. A. Stewart for valuable technical assistance. This work was supported by a research grant from the former Governor of the United Newcast upon Tyne Hospitals.

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