LACK OF HISTOLOGICAL EVIDENCE OF VITAMIN D ABNORMALITY IN THE BONES OF ANEPHRIC PATIENTS

PH. J. BORDIER, S. TUN CHOT, J. B. EASTWOOD, A. FOURNIER AND H. E. DE WARDENER

Centre André Lichtwitz, I.N.S.E.R.M. (U-19), Hôpital Lariboisière, Paris, Xème, France, and Department of Medicine, Charing Cross Hospital Medical School, London

(Received 29 June 1972)

SUMMARY

1. Iliac bone biopsies were obtained from nine patients 3 months to 6 years after bilateral nephrectomy.
2. In eight of the nine patients no histological evidence of vitamin D abnormality was seen in the bones after bilateral nephrectomy.
3. One patient showed histological evidence of osteomalacia before bilateral nephrectomy which improved but did not disappear after bilateral nephrectomy.
4. The biopsies of all nine patients showed evidence of hyperparathyroidism.
5. It is concluded that normal bone mineralization can take place in the absence of the kidneys.

Key words: bone mineralization, nephrectomy, vitamin D.

The histological appearances of the osteomalacia of chronic renal failure are identical to those of vitamin D deficiency (Matrajt, Bordier & Hioco, 1967a) and the changes can be reversed by the administration of very large amounts of vitamin D or of 25-hydroxycholecalciferol (Eastwood, Bordier & de Wardener, 1971). Nevertheless serum anti-ricketic activity in untreated chronic renal failure is normal (Lumb, Mawer & Stanbury, 1971).

Vitamin D is converted in the liver into 25-hydroxycholecalciferol (Blunt, DeLuca & Schnoes, 1968; Blunt & DeLuca, 1969), and further hydroxylations are known to occur. DeLuca and his colleagues identified 21,25-dihydroxycholecalciferol (Suda, DeLuca, Schnoes, Ponchon, Tanaka & Holick, 1970a) and 25,26-dihydroxycholecalciferol (Suda, DeLuca, Schnoes, Tanaka & Holick, 1970b). Fraser & Kodicek (1970) and Lawson, Fraser, Kodicek, Morris & Williams (1971) reported the presence of 1,25-dihydroxycholecalciferol. The most potent of these substances in relation to the intestinal absorption of calcium is 1,25-dihydroxycholecalciferol, the biological activity of which is twice that of 25-hydroxycholecalciferol and three times that of cholecalciferol (Kodicek, Lawson & Wilson, 1970). In contrast
the anti-ricketic activity of 1,25-dihydroxycholecalciferol is less than half that of 25-hydroxycholecalciferol (Omdahl, Holick, Suda, Tanaka & DeLuca, 1971). 25-Hydroxycholecalciferol is converted into 1,25-dihydroxycholecalciferol only in the kidney (Fraser & Kodicek, 1970; Lawson et al., 1971); after bilateral nephrectomy, rats (Fraser & Kodicek, 1970; Lawson et al., 1971) and man (Mawer, Backhouse, Lumb & Stanbury, 1971; Schaefer, Von Herrath & Stratz, 1972) are unable to produce this metabolite. Fraser & Kodicek (1970) proposed that in chronic renal failure the disturbance of vitamin D metabolism is an inability of the kidney to convert 25-hydroxycholecalciferol into 1,25-dihydroxycholecalciferol.

To investigate this hypothesis iliac bone biopsies from nine patients whose kidneys had been removed 3 months to 6 years previously were examined for evidence of abnormality of vitamin D metabolism. In four of these patients bone biopsies were taken before and after the removal of the kidneys in order to study the effect of bilateral nephrectomy on the bones. All nine patients were on maintenance haemodialysis. None had received vitamin D. In eight of the nine patients no histological evidence of vitamin D abnormality was seen in the bones after bilateral nephrectomy. One patient showed histological evidence of osteomalacia before bilateral nephrectomy which improved, but did not disappear after bilateral nephrectomy. All nine patients showed evidence of hyperparathyroidism.

**METHODS**

**Patients**

Nine patients were the subject of this study (Table 1). All were suffering from terminal renal failure, and were treated by maintenance haemodialysis. Patients 1–4 were treated in Paris at Broussais Hospital using twin-coil dialysers for 6 h three times a week. The concentration of calcium in the dialysis fluid was 6.3 mg/100 ml. Patients 5–9 were treated in London at Fulham Hospital using modified Kiil dialysers with Cuprophane membranes (PT 150) for 14 h twice a week. The concentration of calcium in the dialysis fluid was 6.0 mg/100 ml. Dietary phosphorus was more rigorously controlled in the patients treated in London than those treated in Paris, as shown by the plasma phosphorus concentrations (Table 1). Patients 1, 3, 5, 6 and 7 occasionally received aluminium hydroxide therapy and patients 8 and 9 had oral calcium phosphate and calcium carbonate intermittently. There was no history of supplemental vitamin D administration before treatment with maintenance haemodialysis was started, and none was given subsequently. Patient 9 underwent a subtotal parathyroidectomy 5 years after bilateral nephrectomy, i.e. 1 year before the bone biopsy.

**Bone histology**

Iliac bone was obtained by using a trephine (Bordier, Matrajt, Miravet & Hioco, 1964) so that a full thickness specimen across the ilium (6 mm diam., 12–14 mm long) was obtained. The design of the trephine allowed a plug of bone wax to be left in the hole from which the bone had been removed. The biopsies were taken into a saline–formaldehyde solution buffered with phosphate (pH 7.0–7.2) and series of undecalcified histological sections approximately 5 μm thick were stained and examined (Matrajt & Hioco, 1966; Matrajt, Bordier, Martin & Hioco, 1967b). The nature of the investigation was explained to the patients before obtaining their consent to the biopsy.

The iliac bone biopsy consists of a cylinder of bone at each end of which there is a layer of
### Table 1. Details of the patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>Start of maintenance haemodialysis</th>
<th>Bilateral nephrectomy</th>
<th>Pre-nephrectomy biopsy</th>
<th>Post-nephrectomy biopsy</th>
<th>Calcium (mg/100 ml)</th>
<th>Phosphorus (mg/100 ml)</th>
<th>Calcium (mg/100 ml)</th>
<th>Phosphorus (mg/100 ml)</th>
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</thead>
<tbody>
<tr>
<td>6</td>
<td>F</td>
<td>31</td>
<td>April 1967</td>
<td>December 1969</td>
<td>February 1971</td>
<td>9.6</td>
<td>5.1</td>
<td>9.8</td>
<td>4.4</td>
<td>9.6</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>46</td>
<td>September 1969</td>
<td>December 1969</td>
<td>January 1970</td>
<td>10.4</td>
<td>5.0</td>
<td>9.8</td>
<td>4.4</td>
<td>9.6</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>29</td>
<td>August 1964</td>
<td>January 1964</td>
<td>October 1964</td>
<td>9.3</td>
<td>5.0</td>
<td>9.8</td>
<td>4.4</td>
<td>9.6</td>
</tr>
</tbody>
</table>

*Patient 9 underwent subtotal parathyroidectomy in 1969.*
compact bone, the external cortex and the internal cortex of the ilium. The region between the cortical plates of compact bone is cancellous bone; in quantitative studies this is known as the total cancellous volume. It consists of bone tissue and marrow spaces. All the cancellous bone was quantified, first, as regards the volumes of various tissues and, secondly, in relation to the subdivisions of the trabecular surface. The total volume of cancellous bone consists of bone tissue and marrow, the former consisting of calcified bone and osteoid. The surface of the trabeculae can be subdivided into a resting surface, a surface covered by osteoid and a surface showing resorption. The surface covered by osteoid can be subdivided further by the presence or absence of a front of calcification in that osteoid lamella lying closest to the trabeculae. In addition the number of osteoclasts/mm² of bone section and the proportion of osteocytic lacunae showing osteolysis were quantified. Osteocytes were examined in both cortical and cancellous bone. In normal subjects, over 95% of osteocytes have a clear-cut margin, and the border between the osteocyte and bone tissue is easily identifiable. A small number of osteocytes (3.9% ± 0.6) have an irregular border which appears to merge with the surrounding bone, and the normal lamellar structure of the bone in these areas may be absent. Microradiography shows that these appearances correspond to a loss of mineral. This phenomenon is defined as osteocytic osteolysis. The amount of osteocytic osteolysis was quantified and expressed as the number of osteocytes showing osteolysis as a percentage of the total number of osteocytes.

Normal values for bone histology
Values obtained from nine normal individuals aged 20–39 years are shown in Table 2.

RESULTS

Bone histology in anephric subjects (patients 1–9) (Table 2)
In eight of the nine patients the histology of the bones was consistent with a normal action of vitamin D in that there was a normal amount of calcification front and a normal volume of osteoid. In the other patient (patient 4), however, there was evidence of vitamin D abnormality in that there was a diminution in the amount of calcification front, though this was less pronounced than it had been before bilateral nephrectomy. All nine patients showed evidence of hyperparathyroidism, i.e. an increased amount of osteocytic osteolysis and/or an increased number of osteoclasts.

Volume of bone tissue. In five patients the volume of bone tissue was within normal limits. In two (1 and 4) it was increased, and in two (5 and 8) it was decreased. Normal values are also given in Table 2.

Osteoid volume (normal value 3.8% ± 1.7). The osteoid volume was normal in all nine subjects with a mean of 4.0% and a range of 1 to 7%.

Trabecular bone surface lined by osteoid (normal value 12% ± 5). The proportion of trabecular surface covered by osteoid was high in six of the nine patients, and normal in the other three. The mean for the nine patients was 31.0% with a range of 15–60%.

Calcification front (normal value 78% ± 7). The amount of calcification front was normal in all but patient 4. The mean for the nine patients was 75.2% with a range of 50–87%.

Trabecular bone surface showing resorption (normal value 15.3% ± 3.8). The amount of osteoclastic resorption was normal in seven of the nine patients. In the other two patients the
Table 2. Quantitative bone histology in the nine anephric patients, nine normal individuals, thirty patients with vitamin D deficiency osteomalacia and in thirteen patients with primary hyperparathyroidism. BN = bilateral nephrectomy. The calcification front is the % of the osteoid lamella lying closest to the bone trabeculae with a calcification front. The values in parentheses are normal values for corresponding decades, expressed as the mean ± SD.

<table>
<thead>
<tr>
<th>Normal Individuals</th>
<th>Bone tissue volume as % of volume of cancellous bone</th>
<th>Osteoid volume as % of volume of bone tissue</th>
<th>% of trabecular bone surface lined by osteoid</th>
<th>Calcification front (%)</th>
<th>% of trabecular bone surface showing resorption</th>
<th>No. of osteoclasts per mm² of bone section</th>
<th>% of osteocytic lacunae showing osteolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>Pre BN</td>
<td>Post BN</td>
<td>Pre BN</td>
<td>Post BN</td>
<td>Pre BN</td>
<td>Post BN</td>
<td>Pre BN</td>
</tr>
<tr>
<td>9</td>
<td>3-8±1-7</td>
<td>12±5</td>
<td>78±7</td>
<td>15.3±3.8</td>
<td>0.01-0.3*</td>
<td>3-9±0.6</td>
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<tr>
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<td>33.4</td>
<td>40.2 (22.1±4-1)</td>
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<td>2</td>
<td>65</td>
<td>60</td>
<td>56</td>
</tr>
<tr>
<td>3</td>
<td>19.9</td>
<td>17.9 (22.1±4-1)</td>
<td>4</td>
<td>3</td>
<td>23</td>
<td>38</td>
<td>83</td>
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<tr>
<td>4</td>
<td>17.8</td>
<td>18.7 (22.1±4-1)</td>
<td>4</td>
<td>3</td>
<td>23</td>
<td>52</td>
<td>76</td>
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<td>5</td>
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<td>9.1 (21.0±4-0)</td>
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<td>7</td>
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<td>8</td>
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<td>8.2 (22.8±4-3)</td>
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<td>23</td>
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<td>85</td>
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<td>9</td>
<td>15.2</td>
<td>22.8±4-3</td>
<td>6</td>
<td>6</td>
<td>22</td>
<td>--</td>
<td>75</td>
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</tbody>
</table>

Vitamin D deficiency osteomalacia

| No. of subjects | 30 | 22.4±3.6 | 14.6±5.5 | 51.7±10 | 27±7 | 27.8±5 | 0.4-0.9* | 15.1±3-2 |

Primary hyperparathyroidism

| No. of subjects | 13 | 25±3-7 | 9.4±2 | 34±8 | 78.5±6 | 27±3-6 | 0.6-8.6* | 16.5±1-4 |

* Quantification performed on only five subjects.
The mean amount of osteoclastic resorption was above the upper limit of normal. The mean for the nine patients was 19%, with a range of 9–28%.

**Number of osteoclasts** (normal value 0·01–0·3/mm² of bone section). The number of osteoclasts was above normal in six of the nine patients; in the other three it was normal. The mean for the nine patients was 0·83 mm² with a range of 0·13–2·0 mm².

**Osteocytic osteolysis** (normal value 3·9% ± 0·6). The amount of osteocytic osteolysis was above normal in all nine patients with a mean of 9·9% and a range of 5·4–15·6%.

**Effects of bilateral nephrectomy on bone histology (patients 1–4) (Table 2)**

The bone biopsies of two of the four patients (1 and 4) before bilateral nephrectomy showed some evidence of abnormality of vitamin D metabolism in that there was a diminished amount of calcification front in the osteoid lamella lying closest to the bone trabeculae. After bilateral nephrectomy the amount of calcification front in both biopsies had risen; to within normal limits in patient 1. The bones of both patients showed evidence of hyperparathyroidism before and after bilateral nephrectomy; both showed an increase in osteocytic osteolysis with an increase in the number of osteoclasts in the bone biopsy of patient 1, but the proportion of trabecular surface showing osteoclastic resorption was normal in both patients. After bilateral nephrectomy the evidence of hyperparathyroidism had not increased.

The bone biopsies of the other two patients (2 and 3) showed no evidence of vitamin D abnormality either before or after bilateral nephrectomy. The biopsies from both patients showed some evidence of hyperparathyroidism both before and after bilateral nephrectomy in that there was an increase in osteocytic osteolysis and an increased number of osteoclasts. The proportion of trabecular surface showing osteoclastic resorption, however, was normal. The extent of hyperparathyroidism was similar before and after bilateral nephrectomy.

**DISCUSSION**

The osteomalacia of vitamin D deficiency is characterized histologically by the presence of an excess of osteoid tissue covering the surface of the bone trabeculae in combination with a diminished front of calcification in the osteoid lamella lying closest to calcified bone (Matrajt et al., 1967a; Adams, Jowsey, Kelly, Riggs, Kinney & Jones, 1967; Bordier, Matrajt, Hioco, Hepner, Thompson & Booth, 1968; Johnson, Riggs, Kelly & Jowsey, 1971; Bordier & Tun Chot, 1972). In hyperparathyroidism also there is an increased amount of trabecular bone covered with osteoid (Miravet, Matrajt, Bordier, Tun Chot, Gruson & Hioco, 1969) but the amount of calcification front is within normal limits (Miravet et al., 1969; Bordier, Tun Chot, Martin & de Sèze, 1971). Osteomalacia is usually accompanied by histological evidence of hyperparathyroidism, therefore it can only be detected when there is a quantitative reduction in the amount of calcification front. If the calcification front is not stained and quantified the presence of osteomalacia can only be inferred by deciding subjectively that the quantity of osteoid is greater than that expected in pure hyperparathyroidism. The histological appearances specific for hyperparathyroidism are an excess of osteocytic osteolysis and increased osteoclastic activity (Bordier & Tun Chot, 1972).

The amount of calcification front was normal in the bones of eight of the nine anephric patients described above, showing that vitamin D was acting normally at the bone level. In one patient, in whom a biopsy was done both before and after bilateral nephrectomy, the first
biopsy showed the appearances of osteomalacia, whereas in the second biopsy, 3 months later, there was a normal amount of calcification front. In the ninth patient osteomalacia was present both at the time of bilateral nephrectomy and in the biopsy taken 10 months later, but it was much less marked in the second biopsy. In the two patients, therefore, in whom osteomalacia was present before bilateral nephrectomy there had been a distinct improvement by the time the second biopsy was taken. The bones of all nine patients showed evidence of hyperparathyroidism of mild degree. In the four in whom a biopsy was done both before and after bilateral nephrectomy there was no consistent difference in the appearances of the two biopsies. In none of them had the hyperparathyroidism worsened but one patient had had a subtotal parathyroidectomy 5 years after bilateral nephrectomy.

These results can be interpreted in two ways. Either lack of evidence of vitamin D abnormality in the bones of the anephric patients is due to some effect of dialysis on bone which is unrelated to vitamin D metabolism, or dialysis in some way overcomes and prevents the abnormality of vitamin D metabolism which is responsible for the osteomalacia of chronic renal failure.

In support of the first hypothesis administration of calcium carbonate to patients with radiological renal rickets produces clinical and radiological improvement over many months (Snodgrass & de Wardener, 1969). Maintenance haemodialysis against a solution containing 6.0 mg of calcium/100 ml when the patient is hypocalcaemic produces a positive calcium balance (Wing, 1968a). Also administration of calcium carbonate to patients with azotaemic osteomalacia increases the absolute amount of calcified bone (Eastwood et al., 1971). Nevertheless, it is unlikely that the normal mineralization of bone found in these anephric patients was due simply to a positive calcium balance during each dialysis. Mineralization of osteoid tissue normally occurs by means of a linear deposition of calcium (the calcification front) that is confined to the osteoid lamella lying closest to the bone trabecula (Matrajt et al., 1967a). In vitamin D deficiency osteomalacia, and in the osteomalacia of chronic renal failure, in both of which there is a diminution in the extent of calcium deposited at this site, treatment with vitamin D causes a return of calcification front in 6 and 28 days respectively (Bordier, Hioco, Rouquier, Hepner & Thompson, 1969). On the other hand, the administration of oral phosphate to patients with vitamin D deficiency osteomalacia (Bordier, Tun Chot, Martin, Queillé & Hioco, 1971) or the administration of oral calcium to patients with chronic renal failure (Eastwood et al., 1971) does not produce a return of calcification front. Instead calcium is laid down in an irregular and haphazard manner throughout the whole thickness of the osteoid mass. In contrast, the bones of all but one of the anephric patients described here showed a normal amount of calcification front. Even more significantly, there was a rise in the amount of calcification front in the bones of the two patients in whom there was evidence of osteomalacia before bilateral nephrectomy.

In contrast if vitamin D and its metabolites are essential for the normal mineralization of bone then the present evidence demonstrates that the necessary metabolites of vitamin D occur in the absence of the kidneys. This conclusion is inconsistent with the hypothesis of Fraser & Kodicek (1970) that 1,25-dihydroxycholecalciferol is the metabolite responsible for mineralization of bone. They demonstrated that, of the identifiable vitamin D metabolites, 1,25-dihydroxycholecalciferol is present in the greatest concentration in bone cells, and that it is synthesized only in the kidney. In support of Fraser & Kodicek's (1970) hypothesis, Mawer et al. (1971) and Schaefer et al. (1972) showed that 1,25-dihydroxycholecalciferol is not detectable in the blood.
of anephric subjects after administration of radioactive cholecalciferol or 25-hydroxycholecalciferol. Fraser & Kodicek's (1970) hypothesis was an elegant explanation of the presence of osteomalacia, and its resistance to vitamin D, in chronic renal failure. The present evidence that the bones of anephric subjects are not osteomalacic suggests that 1,25-dihydroxycholecalciferol is not necessary for normal bone mineralization in man. 1,25-Dihydroxycholecalciferol could be made locally in bone, but this is unlikely, because although Weber, Pons & Kodicek (1971) demonstrated that 1,25-dihydroxycholecalciferol accumulates in bone-cell nuclei, Fraser & Kodicek (1970) showed that ricketic chick epiphysis in vitro is unable to convert 25-hydroxycholecalciferol into 1,25-dihydroxycholecalciferol. Another unlikely possibility is that anephric patients have large stores of 1,25-dihydroxycholecalciferol and that, for example, these stores were sufficient for 6 years (patient 9).

If normal bone mineralization can occur in the absence of 1,25-dihydroxycholecalciferol, it is possible that cholecalciferol and its other metabolites are directly responsible for bone mineralization. Two of these metabolites, 21,25-dihydroxycholecalciferol and 25-hydroxycholecalciferol, and cholecalciferol itself, occur in the nuclei of bone cells and all three of these substances have an effect on bone mineralization that is more marked than that of 1,25-dihydroxycholecalciferol (Suda et al., 1970a; Omdahl et al., 1971; DeLuca, 1971). It is still difficult to explain the development of osteomalacia in patients with chronic renal failure before maintenance haemodialysis. Lumb et al. (1971) demonstrated that patients with chronic renal failure have a normal capacity to synthesize 25-hydroxycholecalciferol and that the anti-ricetic activity of the serum is normal. Perhaps uraemic serum interferes with the normal mechanism of bone mineralization. This is supported by the fact that osteomalacia is improved by maintenance haemodialysis. In contrast, maintenance dialysis does not improve the intestinal absorption of calcium (Wing, 1968b; Stanbury, 1972). It is this abnormality which is most likely to be related to the impaired ability of patients with renal disease to produce 1,25-dihydroxycholecalciferol. Wong, Norman, Reddy & Coburn (1972) showed that administration of 1,25-dihydroxycholecalciferol to uraemic rats stimulates the intestinal absorption of calcium, and Omdahl et al. (1971) showed that 1,25-dihydroxycholecalciferol is the vitamin D metabolite which causes the greatest increase in intestinal absorption of calcium.

REFERENCES

Bone histology in anephric patients


