BONE FORMATION IN HYPERCORTISONISM

By

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ABSTRACT

Bone formation has been reported to be both low and high in hypercortisonism. The present report describes our efforts to elucidate this controversy. Five patients with hypercortisonism were given tetracycline to evaluate both the level and the rate of formation of new bone tissue. Specimens of bone were taken for biopsy. Measurements showed that the rate of formation is normal but the level is low, while resorption of bone is elevated. The study thus established that the abnormality of bone turnover in this disorder is of a depressed level of formation accompanied by increased resorption.

Hypercortisonism frequently is associated with osteoporosis, which may develop with remarkable rapidity (Howland et al. 1958; Iannaccone et al. 1960; Sprague et al. 1956). The cause of loss of bone has been the subject of both experimental studies and investigations in man. Early studies by Sissons (1956) suggested that resorption was normal in patients with Cushing's syndrome (Sissons 1956, 1960), while experimental evidence suggested that an increase in resorption of bone was the main feature of administration of cortisol to rabbits and rats (Raffo 1960; Storey 1957, 1961). Increased resorption has been the common experience of later investigators, both in experimental studies (Adams & Jowsey 1967) and in man (Jowsey 1966; Riggs et al. 1966), although whether this is the direct effect of cortisol or is mediated through the parathyroids remains controversial (Collins et al. 1962).

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Until recently, all available information on formation of bone in hypercorticosteroids suggested that it was decreased. This finding was the experience of Storey (1957, 1961) in animals, Frost & Villanueva (1961), Sissons (1960), Jowsey (1966), and Riggs et al. (1966) in man, and from direct observations on bone tissue from humans with hypercorticosteroids. Less direct evidence of a depressed formation comes from \(^{47}\text{Ca}\) behaviour in rats given cortisone (Milhaud et al. 1960) and from studies of the wound healing and fracture healing in Cushing's syndrome (Sissons 1956; Plotz et al. 1952). In 1967, a report appeared in which bone formation, measured by single-dose tetracycline labeling, was normal or even elevated in a comparatively large series of patients with hypercorticosteroids (Birkenhäger et al. 1967). An increase in the number of osteoblasts also was reported. Since this directly contradicted our previous findings, we wished to reinvestigate carefully the level of depressed bone formation in this disorder using other criteria in addition to quantitative microradiography, which we used in our original reports (Jowsey 1966; Riggs et al. 1966).

**REPORT OF CASES**

Of the five patients studied, three had exogenous and two had endogenous Cushing's syndrome. Four of five patients received two oral labels of tetracycline as markers for bone formation. One patient (case 1) received only one oral dose of tetracycline.

Case 1. — A 61-year-old white man was admitted to the Section of Dermatology of the Mayo Clinic in April 1968 with the diagnosis of bullous pemphigoid of the mouth and throat. He had taken 20 to 30 mg of corticosteroids daily for 1 year in the form of prednisone, and at the time of his admission, he was taking 30 mg daily. Since falling down a flight of stairs in January 1968 he had had chronic pain in the dorsal region of the back. The only other medical condition was mild diabetes mellitus. Physical examination showed moderate evidence of Cushing's syndrome. Serum values were as follows: calcium 9.6 mg/100 ml, phosphorus 3.4 mg/100 ml, and alkaline phosphatase 49 U/liter. Roentgenograms of the spine showed diffuse osteoporosis with compression fractures of thoracic vertebrae 6 through 10 and 12 and lumbar vertebra 1. Biopsy of the left eighth rib in the anterior axillary line was done on May 2, 1968. A single oral dose of 750 mg of tetracycline was given on April 21, 1968.

Case 2. — A 37-year-old white woman was seen at this clinic in November 1968. She had a 1-year history of mild Cushing's syndrome. The urinary 17-ketosteroids measured 7.9 mg/24 h and the ketogenic steroids 17.9 mg/24 h. The blood corticosteroids measured 20.8 and 15.7 mg/100 ml and failed to suppress with 2 mg daily of dexamethasone over 3 days. While the patient was receiving metyrapone (Mepiprone, 500 mg every 4 h), the ketogenic steroids increased to 61 mg/24 h. She had never had pain in her back. The serum values were 9.3 mg/100 ml for calcium, 3.3 mg/100 ml for phosphorus, and 33 U/liter for alkaline phosphatase. Roentgenograms
of the spine showed evidence of diffuse osteoporosis but no compression fractures of the vertebrae. The patient had undergone partial gastrectomy for duodenal ulcer in February 1966 and hysterectomy in March 1968; on November 26, 1968, bilateral total adrenalectomy was done and hyperplastic adrenal glands weighing 17.3 g were removed. A specimen of the right sixth rib in the anterior axillary line was obtained for biopsy at the time of this operation. A single oral dose of 750 mg of tetracycline was given on November 10 and November 24. She was not aware of having recently received tetracycline drugs.

Case 3. — A 65-year-old white man with a history of severe chronic asthmatic bronchitis of 15 years' duration and of duodenal ulcer and hypertension was seen at this clinic in June 1968. Since 1961, except for brief intermittent periods, he had taken corticosteroids in the form of triamcinolone (6 to 12 mg daily). Approximately 2 months before his examination at this clinic, the use of triamcinolone had been discontinued and he had taken 20 mg of prednisone daily. He reported that during the preceding 6 months he had experienced several acute episodes of pain in the back. Physical examination showed evidence of mild Cushing's syndrome. Serum values were 9.2 mg/100 ml for calcium, 4.1 mg/100 ml for phosphorus, and 42 U/liter for alkaline phosphatase. Roentgenograms of the spine showed evidence of diffuse osteoporosis with compression fractures of thoracic vertebrae 5, 6, 7, and 12. In July 1968, a specimen for biopsy was obtained from the left eighth rib in the anterior axillary line. Single oral doses of tetracycline (750 mg) were given on June 27 and July 28, 1968. In past years he had taken many antibiotics for respiratory infections.

Case 4. — A 48-year-old white woman was admitted to the Section of Dermatology of the Mayo Clinic in April 1968 with the diagnosis of pemphigus vulgaris, which had been present since October 1964. Since the onset of her disease she had taken corticosteroids daily in the form of prednisone (10 to 60 mg), and at the time of her admission she was taking 55 mg daily. She denied having significant back pain. Physical examination showed moderate Cushing's syndrome. The value for serum calcium was 10 mg/100 ml, phosphorus 2.5 mg/100 ml, and alkaline phosphatase 25 U/liter. Roentgenograms of the spine showed evidence of mild osteoporosis without vertebral compressions. Two healing fractures of the ribs were present. On April 26, biopsy of the right seventh rib in the anterior midaxillary line was obtained. She had taken tetracycline (250 mg twice daily) from March 1 to 15, 1968, and had received another oral label of 750 mg of tetracycline on April 25.

Case 5. — A 37-year-old white woman was seen at this clinic in March 1969 with a 1-year history of mild Cushing's syndrome. On examination, the physical findings were compatible with this diagnosis. In urine collected over 24 h the 17-ketosteroids measured 7.9 mg and the 17-ketogenic steroids, 11.7 mg. The value for corticosteroids was 29.5 μg/100 ml of blood at 8 a.m. and 27.0 μg/100 ml at 4 p.m. A daily dose of 2 mg of dexamethasone over 3 days failed to bring about suppression. While she received 500 mg of Metopirone every 4 h, the ketogenic steroids increased to 70.8 mg/24 h. She never had had back pain. Roentgenograms of the spine and skull were without evidence of osteoporosis. Radiation of the pituitary gland was the treatment of choice. On April 23, 1969, local anaesthesia was used and a specimen from the left sixth rib was obtained for biopsy. The patient was given single oral doses of 700 mg of tetracycline on March 18, April 16, and April 22. She was not aware of the recent administration of tetracycline drugs.
METHODS

A specimen for biopsy was taken from a rib 24 h after the second tetracycline label had been given. It was fixed in alcohol, embedded in methacrylate, and cut into 100 µm sections. Microradiograms were then made. Thereafter, the calcified section was ground to 70 µm and stained with Paragon (Martin et al. 1966). From the microradiogram, bone formation and resorption were measured (Jowsey 1966). Any area that showed formation was checked with the calcified section to see if osteoid tissue and osteoblasts, both of which are indicators of newly formed tissue, also were present in that area. In the sections viewed with ultraviolet light, the length of each single band and of each double band (most important) was measured. The measurement for the two types of bands were kept separate. In addition, the width of tissue between any double bands of tetracycline was measured, and this distance was divided by the number of days between administrations of tetracycline. In the patient receiving only one dose of tetracycline, the width of tissue was measured between the tetracycline band and the calcification front. The scanned sections also were carefully inspected for other surfaces showing formation of new bone by the presence of unmineralized osteoid and osteoblasts.

RESULTS

Table 1 demonstrates clearly that bone formation is indeed reduced in hypercortisonism. On the microradiograms, bone formation, in the few instances in which it occurred, was always associated with darkly staining osteoid of normal width, osteoblasts, and a double band of tetracycline (Fig. 1). In all sections, bands of tetracycline appeared on bone surfaces that were not in the process of formation according to any of the four criteria for new tissue deposition, namely, the appearance of osteoid tissue, osteoblasts, a border of low density seen in the microradiogram, and a double band of tetracycline. Such single labels represented artifacts, that is, surfaces of bone that lay at right angles to the surface of the section and produced a smudged-out area of fairly low intensity resulting from uptake on sclerotic surfaces. These surfaces, even when they are perpendicular to the surface of the section, occasionally retain tetracycline at a level that produces a clear band; the uptake corresponds, in such instances, to a line of increased mineral density on the bone surface that occurs when formation stops. During this process, tetracycline is incorporated into the bone tissue and remains.

The majority of single bands of uptake were the result of tetracycline previously administered for therapeutic reasons. These bands generally are buried in the bone but may appear on the bone surface. They are usually narrow, single lines, but in one patient a broad band appeared as a result of continuous intake of tetracycline over a few weeks (case 4) (Fig. 2). The rate of
Table 1.
Morphological features in 5 cases of hypercortisonism.

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (years) and sex</th>
<th>Corticosteroid history</th>
<th>Bone formation, % total surface</th>
<th>Tetracycline double band, % total surface</th>
<th>Rate of bone formation, μm/day</th>
<th>Tetracycline single band, % total surface</th>
<th>Bone resorption, % total surface</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>61 M</td>
<td>Exogenous 1 year</td>
<td>0.2</td>
<td>0.2</td>
<td>1.1</td>
<td>3.8</td>
<td>21.5</td>
</tr>
<tr>
<td>2</td>
<td>37 F</td>
<td>Cushing's syndrome</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.4</td>
<td>21.5</td>
</tr>
<tr>
<td>3</td>
<td>65 M</td>
<td>Exogenous 7 years</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>14.9</td>
</tr>
<tr>
<td>4</td>
<td>48 F</td>
<td>Exogenous 4 years</td>
<td>0.4</td>
<td>0.4</td>
<td>1.7, 1.2</td>
<td>0.9</td>
<td>7.2</td>
</tr>
<tr>
<td>5</td>
<td>37 F</td>
<td>Cushing's syndrome</td>
<td>0</td>
<td>0.5</td>
<td>0.8, 0.7, 0.7</td>
<td>2.2</td>
<td>9.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age, years</th>
<th>Normal value ± sd</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Formation, %</td>
</tr>
<tr>
<td>40</td>
<td>2.2 ± 1.4</td>
</tr>
<tr>
<td>60</td>
<td>3.1 ± 2.0</td>
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</tbody>
</table>
Fig. 1 (case 2).
Microradiogram (Left, ×75) and view of tetracycline band (Right, ×50) in rib. A clear double label of tetracycline on the bone surface (Right) appears in the microradiogram (Left) as bone of low density. A double label in an adjacent osteone is buried deep within the bone and is present as a result of previous uptake of tetracycline.

Bone formation per unit of surface in the two patients in whom formation was noted was 1.2 µm and 2.1 µm/day, respectively.

Bone resorption tended to be high in all patients.

**DISCUSSION AND CONCLUSIONS**

Despite an accurate history of a patient’s past treatment, it is sometimes impossible to be certain whether or not tetracycline has been administered. As a result, any measurement in which the uptake of tetracycline on a bone surface is taken as an indication of new bone formation must include all areas where this substance has labeled the bone previously and has not been buried by further deposition. Nowadays, one rarely finds a patient who has not had tetracycline at some previous time, so that evaluation of bone formation by
The majority of tetracycline bands result from previous administration over a relatively long period. A single band, resulting from the dose administered during the patient’s stay at the Mayo Clinic, is bordered by osteoid (barely visible, Right) and is an area of low mineral density in microradiogram (Left).

Uptake of tetracycline is fraught with hazards. It is comparatively simple, however, to relate the bright tetracycline band with some other criterion of bone formation, such as the presence of unmineralized osteoid in a calcified section or a low density area in a microradiogram. Osteoblasts are further evidence of bone formation although they are somewhat less reliable since, after active tissue deposition has stopped, they are often still present (albeit looking more and more like fibroblasts) when other criteria of formation have disappeared. This seems to be particularly true in hypercortisonism. Also, the presence of osteoid as an indication of bone formation, although simple and generally reliable, is misleading in any form of osteomalacia because tissue deposition may cease and the failure of calcification may preserve a border of uncalcified tissue for a long time.

The use of two labels, separated by an interval of at least 7 days, has the
advantage of providing unquestionable evidence of deposition of new tissue. If surface of the bone is still covered with osteoid tissue, then the tissue must have been formed recently and is still being deposited. The present study illustrates some of the problems of using tetracycline alone to label new bone formation, explains the reason for the controversy concerning the level of bone formation in hypercortisonism, and demonstrates that in hypercortisonism, bone formation is indeed depressed and, as previously suggested, in conjunction with the elevated resorption levels the loss of bone is rapid.

REFERENCES


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