OSTEOPOROSIS: A NECROPSY STUDY OF VERTEBRAE AND ILIAC CRESTS

ALAN VOST, M.B., CH.B.

From the Department of Pathology, Montreal General Hospital, Montreal, Canada

Osteoporosis is a diminution of hard bone substance without obvious changes in its chemical or histologic composition. Generalized osteoporosis is evident most readily in those bone regions which are predominantly cancellous, such as the vertebrae, sacrum, sternum, ribs and iliac crests. While osteoporosis may be secondary to other diseases, as in thyrotoxicosis and Cushing's syndrome, the etiology, for the most part, is obscure.

Study of this disease has been hindered by the difficulty in its quantitative assessment. It has been shown that the calcium content of bone salt is remarkably constant,[1] and the expression of calcium weight per unit volume of bone remains the parameter by which other measurements should be judged.

Radiologic examination of vertebral bone slabs has been closely correlated with calcium analysis by Caldwell and Collins in 100 necropsied patients.[2] The normal interposition of soft tissues has been shown by Fusi [3] to require the extraction of 60 per cent of calcium from vertebrae before marked radiologic changes appear. For clinical purposes there have been developed methods using ratios of cortical thickness to shaft diameter of tubular bones and of anterior to central vertebral body heights.[4]

Bone biopsy using trephined material from the iliac crest has been graded visually by Beck and Nordin, as an index of osteoporosis.[5] The crude assessment of vertebrae at necropsy by visual examination and finger pressure is unreliable, and the histologic examinations of routine bone sections is also suspect.

The purpose of the present study was to examine the vertebrae and iliac crests in a random necropsy series using a histologic method with a minimum of subjective interpretation and one which would show a significant correlation with vertebral calcium concentration.

MATERIAL

Ninety-two random necropsies in a general hospital were utilized. In all of these the fourth lumbar vertebra was removed, and in 22 an iliac crest sample was taken approximately 2.5 cm. behind the anterior superior spine.

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METHODS

After removal of soft tissues, the bones were stored, frozen, for a minimum of 2 weeks. Nine vertebrae showing symmetrical bodies were selected to demonstrate a wide spectrum of normal and osteoporotic bone. Each of the 9 was vertically bisected and one half submitted for chemical analysis and the other half for histologic examination.

Histologic Methods

Through the center of 92 vertebrae, an antero-posterior slab of bone of approximately 3 to 4 mm. thickness was cut by a fret saw, and, similarly, 2 mm. slabs were taken from the portions of iliac crest. These sections were agitated in a 10 per cent aqueous solution of methyl alcohol for 32 hours with 2 changes of solution. In this way the marrow stroma was destroyed, and sections were then washed under cold tap water to remove marrow residuum. After fixation in 10 per cent formol saline, decalcification was carried out in a formic acid and citrate solution for 6 days. The tissues were then embedded in paraffin, and a minimum of 2 sections were cut at 50 μ from each block by a rotary microtome. The sections were stained with Heidenhain's iron-hematoxylin method and differentiated with microscopic control to delineate any residual soft tissue. In this manner thick sections of the complete transverse area of vertebrae and iliac crests, showing bone as a dense black network, were obtained (Fig. 1). Sections were used for photometry after inspection for consistency and absence of artifact.

Microscopic examination using a constant light source was then performed with a Leitz microscope with oblique visual and vertical tube heads. Attached to the latter was a photometer and, with a medium power (10:1) lens, this was adjusted to read 100 per cent on clear sections of each slide. Photometer readings were then taken in adjacent fields so that each vertebral section was completely scanned by approximately 50 circular fields, 3 mm. in diameter. The outer limit of scanning was the external aspect of the vertebral cortex. The mean value of all readings for each vertebra was then subtracted from 100 to estimate the percentage of light blocked by the trabeculae. The logarithm to base 10 of the latter value was found and described as the Photo Index. This index was therefore a logarithmic expression of the percentage area of bone tissue in the vertebral sections. In similar fashion the iliac crests were read, using 2 adjacent fields to include the midportion of the superior cortex.

The consistency of the method was verified by the similarity of photo indexes in serial sections of the midportions of several vertebrae.

Decalcified bone sections stained with hematoxylin and eosin were examined from adjacent vertebrae in all cases. In particular, the presence of osteoid seams and Paget's disease was sought. Two vertebrae, one with severe Paget's disease and the other with tumor metastasis, were rejected from the series.

Chemical Methods

The 9 vertebrae previously selected were vertically bisected and immediately a fine coating of paraffin wax was placed over the exposed cancellous surface of one half. The volumes of these halves were found by water displacement. The bones were then fragmented and dried at 100° C. Fat was removed over boiling ether, and the dried, defatted bone was weighed and its weight expressed in gm. per 100 ml. of vertebra. The dried, defatted bone (500 mg.) was then ashed at 600° C. for 48 hours and the ash analyzed for calcium by an oxalate method adapted after that of Shohl and Pedley.[6] The total calcium and calcium concentration were then found for each hemivertebra.

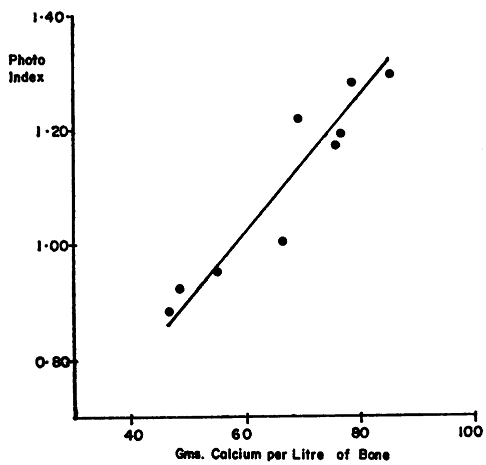
RESULTS

The photo indexes of the analyzed vertebrae are shown in Table I in relation to the calcium concentrations in volume of vertebrae and in weight of dried defatted bone. The relationship of photo indexes to calcium concentration per liter of vertebral bone (Text-fig. 1) was linear and statistically significant ($chi^2 = 2.01$, d.f. = 7, $P > 0.95$) and an even closer linear relationship was shown between photo indexes and the percentage of dried defatted bone in the vertebrae ($chi^2 = 0.053$, d.f. = 7, $P > 0.99$).

TABLE I

RELATION OF PHOTO INDEXES OF ANALYZED VERTEBRAE TO CALCIUM CONCENTRATIONS

Photo index	Gm. calcium per liter of vertebral bone	Gm. equivalents calcium per kg. of dried, defatted bone	Gm. wt. of dried, defatted bone per 100 ml. of vertebra
1.293	85.6	9.15	46.8
1.279	79.34	9.02	44.0
1.190	76.86	9.02	42.6
1.172	75.98	8.87	43.1
1.216	69.52	8.63	39.5
1.021	66.9	8.82	38.0
0.947	54.2	8.63	31.5
0.922	48.6	8.30	29.0
0.884	47.0	8.45	27.7

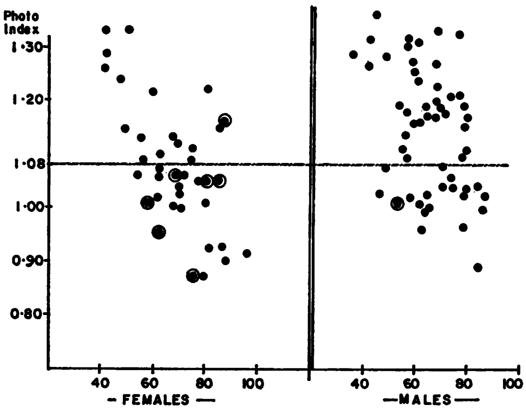


TEXT-FIG. 1. Relationship of photo indexes to calcium content of vertebral bone.

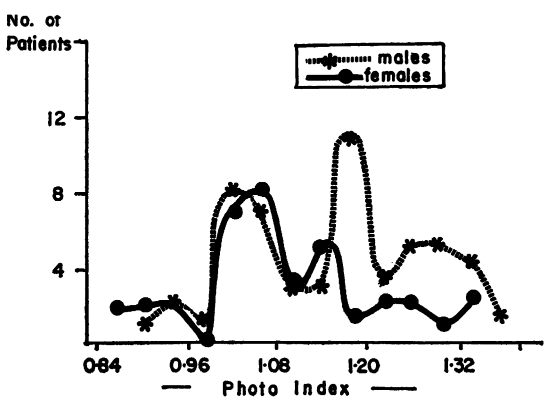
The photo indexes for all vertebrae were graphed against age for male and female patients (Text-fig. 2). The tendency to lower vertebral bone densities with age is shown in both sexes. There was a greater frequency of low readings in the female group and they occurred at a much earlier age than in the males. Those patients with a history of fracture with minor trauma were shown to have vertebral photo indexes under 1.08, with the exception of one woman having osteomalacia with massive osteoid seams, and therefore a falsely high photo index.

Population frequency graphs showed two major peaks (Text-fig. 3), and the distribution was remarkably similar in both sexes. The differentiation of the two major groups lay at a photo index of 1.010 in both sexes. A small secondary peak was present in the denser bones.

The distribution of emaciated patients between the groups with vertebrae of a high photo index (> 1.22) and those of low index (< 0.98) was



TEXT-FIG. 2. The variation of photo indexes with age. Those encircled had bone fractures without severe trauma.



TEXT-FIG. 3. Photo index of bone tissue content of vertebrae by sex distribution.

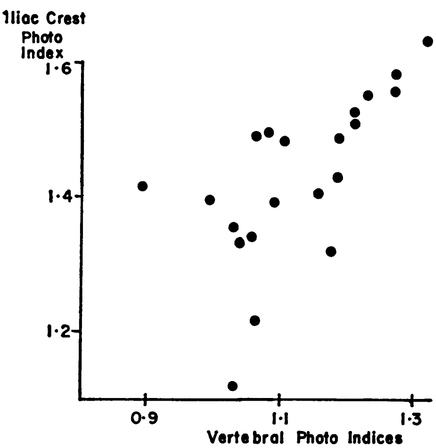
not statistically significant. Patients older than the mean age for the series composed most of this low index group, whereas the high index group was almost entirely of patients below the mean age.

Iliac crest indexes were graphed against vertebral indexes, and the correlation of the two readings was poor, with a wide scatter in the lower range (Text-fig. 4). Indexes of the cancellous bone of iliac crests were also taken, but they showed very poor correlation with the vertebrae.

It appeared from the population frequency graphs that a photo index of 1.010 was the point at which the groups of normal and osteoporotic vertebrae overlapped. Using this figure, 59 per cent of the females and 36 per cent of the males in this series showed osteoporosis of the fourth lumbar vertebra.

Discussion

Osteoporosis has attracted increasing attention in recent years. Its definition as diminution of hard substance of bone without obvious changes in its quality is based on histologic assessment. Osteoporosis shows its most striking effects in those bones which are largely cancellous, and bone ash content of human cadavers shows little variation except in predominantly cancellous bones.[7] The remarkable metabolic activity of bone appears to depend partially on the surface area of hard bone sub-



TEXT-FIG. 4. Comparison of photo indexes of iliac crests with vertebral indexes.

stance exposed to ionic movement and, thus, cancellous bone is more active than compact bone. Arnold [8] has shown less ash and reduced mineralization in the trabecular bone as compared to the compact bone of the same vertebrae. Variations in the organic matrix, particularly in the mucoproteins, have been described in osteoporotic bones.[9]

Studies of vertebral weights [10] and bone ash content [7] have shown no significant decrease in these values with age.

The assessment of osteoporosis, in this series, by vision and finger pressure examination of the gross vertebrae was quite unreliable, and the histologic features of the conventional hematoxylin and eosin-stained sections were often misleading. Biconcavity of the vertebrae was not a reliable guide to the extent of porosis but loss of horizontal trabeculation was of value.

Radiologic methods were not used in this study, but Caldwell and

Collins [2] showed a good regression correlation between vertebral calcium concentration and step wedge readings of vertebral radiographs. The difficulties of radiologic estimate of osteoporosis in the living patient with soft tissue interposition is notorious.

Ingenious methods of visual scanning in iliac crest trephine biopsy specimens have been attempted,[5] but in this study of larger areas of iliac crest, the correlation with vertebral indexes showed wide scatter. This may partially be accounted for by the variable skeletal distribution of osteoporosis, but many sections showed localized thickenings in the superior cortex, and readings from adjacent areas gave quite variable results. However, cortical thickenings tend to disappear in osteoporosis, and biopsy may be a useful assessment in severe osteoporosis.

Calcium expressed as weight per unit value of bone is, at present, the best index of osteoporosis. This method was used to judge the accuracy of taking thick (50 μ) vertebral sections and estimating the light blocked by the hard bone structure. The former is a volumetric and the latter an area expression, and they do not give a linear correlation. The logarithmic expression of the percentage of blocked light was therefore used.

The results of calcium estimations and other values of bone chemistry in this series were subject to the inaccuracies of hemisection of vertebrae and the use of wax in coating the cut surface.

The removal of all cellular material was difficult in extremely dense bone and in slabs which were too thick. However, differentiation of Heidenhain's stain markedly reduced the darkness of cellular material without substantially altering bone stains. The method was very suitable for normal and porotic bone.

The correlation of photo indexes and calcium content was very high and the method was of a sensitivity such that a change of 0.04 in the photo index was equivalent to a change of 4 mg. in calcium content. By taking the frequency of groups with 0.04 increments in photo indexes, it was possible to show in the male and female populations 2 sharply differing peaks separated by the equivalence of only 8 and 16 gm. of calcium per liter. The point of differentiation (photo index of 1.010) corresponded to 67.6 gm. $\pm$ 0.6 gm. per liter, and it was of interest that 3 female patients with hip fractures after minor trauma showed photo indexes equivalent to 62 to 64 gm. of calcium per liter of vertebral bone.

It was not possible to demonstrate a clear effect of age on osteoporosis although the disease was more common with increasing age. Neither, because of the small number in the group, was it possible to estimate the effect of other diseases.

The failure to demonstrate correlation between osteoporosis and body nutrition was probably a reflection of the crude methods of assessment of nutrition, and it is quite possible that osteoporosis may have a relation to the more meaningful physiologic measure of lean body mass rather than total body weight.

It appeared from this study that osteoporotic and normal vertebrae formed a bi-modal population and that osteoporosis was not merely the less dense bone in a normally distributed population. It was also possible to estimate the differentiation region of photo indexes and calcium concentration and, with some clinical support for these figures, one could state that lumbar vertebrae containing less than 67 gm. of calcium per liter (by the method of Shohl and Pedley [6]) were probably osteoporotic.

It was further apparent that osteoporosis was more common in this necropsy population than previous estimates suggested,[5] although the present sample was not large. This was probably because previous authors had to confine themselves to a study of severe osteoporosis.

Summary

In a random series of 90 necropsies, sections of lumbar vertebrae were examined after removal of marrow. The bone trabeculae were stained black, and the sections were measured by a photometer to obtain an estimate (the photo index) of the hard bone area in each vertebra. Nine vertebrae were selected for calcium analysis and these showed a satisfactory correlation of photo indexes with calcium concentrations.

Study of the population frequency graphs suggested a bi-modal population of normal and osteoporotic vertebrae, and vertebrae containing less than 67 gm. of calcium per liter were probably osteoporotic. The percentage of osteoporosis in this series (59 per cent of females and 36 per cent of males) was higher than previous estimates.

The degree of porosis in iliac crests and vertebrae appeared crudely related by this method.

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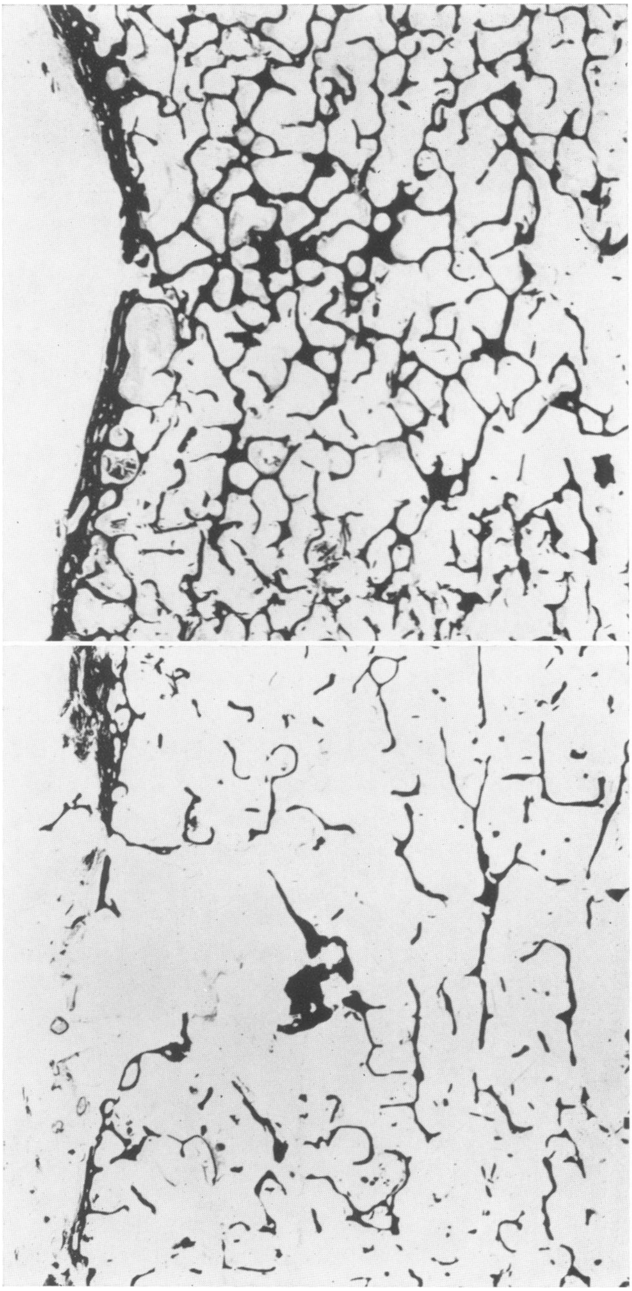
LEGENDS FOR FIGURES

FIG. 1. Comparison of identical areas of vertebrae with Heidenhain's stain of (A) photo index 1.29 and (B) photo index 0.86. × 5.7.



1A

1B