ASSESSMENT OF VERTEBRAL OSTEOPOROSIS
BY RADIOGRAPHIC AND CHEMICAL METHODS POST-MORTEM

R. A. CALDWELL and D. H. COLLINS, SHEFFIELD, ENGLAND

From the Department of Pathology, University of Sheffield

The measurement of changes brought about by disease is an important step in understanding it, enabling us to determine its incidence and severity under various conditions. A quantitative measurement of osteoporosis is however not easy to obtain. In life a useful histological assessment can be made with care from bone biopsy (Beck and Nordin 1960), but from only a limited field of bone tissue. As ordinarily practised, radiography reveals only advanced degrees of osteoporosis and allows of no accurate comparison of case with case. Barnett and Nordin (1960) have recently recommended indices based upon cortex to shaft ratio in tubular bones and on biconcavity of vertebral bodies. Radiological methods for calculating bone density tend to be elaborate or inaccurate. Although some of the disabilities have been overcome in radiographing peripheral limb bones (Jackson 1951, Virtama 1957; Keane, Spiegler and Davis 1959), the interspersed soft tissue, the position of the bones relative to the tube and the film, and variation in their size are factors that especially prejudice accurate observations on the vertebrae. Even in the post-mortem room, osteoporotic bone atrophy cannot be gauged by the direct measurement of the size and weight of the bones, because the essence of osteoporosis is a rarefaction—that is, a reduction of calcified bone mass in a unit volume of the anatomical bone.

In osteoporosis the trabeculae of cancellous bone become unduly slender and many vanish; those that remain are widely separated. Compact cortical bone undergoes cancellous transformation by the progressive enlargement of resorption cavities which become filled with marrow tissues. The histological picture of osteoporosis indicates an atrophy of the bony framework unaccompanied by osseous regeneration, by fibrous substitution or by notable osteoclastic reaction, as Hadfield observed in reporting on the histological sections in the cases of Burrows and Graham (1945). Osteoporosis has been attributed to the failure of osteoblasts to restore bone lost in the process of physiological resorption (Albright 1947), but Fraser (1959) found no evidence from clinical tests so far available of defective laying down of bone in osteoporosis, and Urist (1958) states that it remains to be proven that the rate of resorption is the same as in normal individuals. Whatever be the case, the bone that remains is calcified bone, but whether individual osteones and trabeculae show abnormally variable degrees of calcification in osteoporosis is a matter yet to be studied by microradiography. Osteoporosis is histologically distinguishable from osteomalacia by the absence of uncalcified osteoid seams with their border of active osteoblasts.

Since we agree with McLean and Urist (1955) that the proper use of the term osteoporosis is to comprehend an increased porosity or "a decrease in the hard portions of bone substance in favour of a relative increase in the soft portions," a measurement of osteoporosis may suitably be expressed in terms of the mass of calcified bone in a certain volume of skeletal structure. For practical purposes this can be reduced to a calculation of the amount of calcium contained in that volume, because the proportion of calcium in bone salt is virtually constant at around 36 per cent (Neuman and Neuman 1958) and it is bone salt that gives strength and rigidity to the skeleton. Before accepting the calcium/volume index as a measure of osteoporosis one must of course be satisfied by histological or other examination that cases of osteomalacia, osteitis fibrosa, Paget's disease of bone, or other bone disease have not been included in the investigation.

Chemical analysis is the direct way of estimating the calcium content of bone. There
are also two indirect methods: 1) the radiographic density, and 2) the specific gravity. In dealing with the vertebrae the radiological method in clinical practice has the inherent difficulties already mentioned and it is, moreover, often impossible to allow for the varying thickness of bone that has been radiographed. In the post-mortem room a uniformly thick slab of bone can be secured and radiographs can be made of it under standard conditions. The specific gravity method was used for some years by one of us (Collins 1959) in the post-mortem room: small cubes of cancellous bone were cut from vertebral centra and dropped into copper sulphate solutions covering a range of specific gravities. The method was considered too crude to continue with, but gave some results in a large series of cases which will be referred to in our discussion.

It is our purpose in the present paper to put forward some results of a study in which the radiographic density and the calcium content of lumbar vertebrae obtained at hospital necropsies have been measured, to indicate the range of calcium contained in these bones, and to show that this can be inferred from the radiograph of the bone slab. Other data relating to the incidence of osteoporosis among persons dying in hospital and the possible association of other morbid conditions have been gathered, but sufficient material has not yet been examined for a detailed analytical report on these lines.

METHODS

Samples of vertebral bone were obtained in a random series of 100 necropsies on persons dying from various diseases at the Sheffield Royal Infirmary.

A one-centimetre-thick parallel-sided vertical slab of lumbar vertebral bodies and intervening discs, generally including the first lumbar to first sacral vertebrae, was obtained in each case by sawing from the front of the spinal column through to the spinal canal, using two hand tenon saws coupled together with the blades one centimetre apart. The saw cuts were made in such a direction as to include the mid-sagittal plane of the vertebral bodies. After light brushing under running water each specimen was briefly immersed in 1 per cent
gelatin and then suspended in neutral formol-saline. Specimens showing infiltration by tumour or severe Paget's disease were excluded from the series. The bones were kept immersed in fluid until a few moments before radiography. When satisfactory radiographs had been obtained chemical and histological methods were proceeded with.

Radiography—Wet bone slabs were radiographed alongside an aluminium step-wedge. This was built of one to ten laminae of aluminium sheet 0.3 millimetre thick stepped in such a way as to give a thickness range of 0.3 to 3.0 millimetres numbered from one to ten step-wedge units (Figs. 1 to 3). Constant exposures of ten seconds at 10 kilovolts and 10 milliamps
were made at a tube distance of 63.5 centimetres. Radiographic films (Ilford 6\(\frac{1}{2}\) x 8\(\frac{1}{2}\) inches) were exposed in plastic cassettes and developed for five minutes without agitation at 20 degrees Centigrade (Kodak 19b developer).

The density of the radiographic image of each fourth lumbar vertebra (one centimetre thick) was estimated photometrically in terms of step-wedge units as shown in Figure 1. Light from an electric lamp (Philips Photocrescenta, 240 volts, 75 watts) was directed through a 2 x 1 centimetre slot of the image of the vertebral body and, on adjusting the film, through each step image of the step-wedge.
The intensity of the light transmitted in each position was measured by a 6-centimetre photo-cell (E.E.L.) connected to a galvanometer (Pye Scalamp). When the vertebral bodies showed patchy variation of density the average of several readings was recorded; care was taken to observe that each step of the wedge gave a proportionate galvanometer deflection, thus ensuring that each film had been exposed and processed correctly within its sensitivity (Hurter-Drifield) range.

Calcium analysis and calcium/volume index—In addition to the total calcium contained in the bone blocks their wet weight and ashed weight were recorded, but the calcium/ash ratio was found to be more or less constant (circ. 38 per cent) in all circumstances and the calcium/wet weight ratio does not comprehend the idea of osteoporosis being a reduction of bone mass in anatomical bone structure. For this it was necessary to measure the volume of the intact bone before ashing; this was done by displacement. After radiography, the slab of the fourth vertebral body was dissected from its neighbours and, with a scalpel, stripped of adherent cartilage, blood vessels and connective tissue. Marrow tissues still protected by a film of gelatin remained undisturbed. The block of vertebra was then blotted to remove surface moisture and suspended on one arm of a balance by a fine nylon thread, where it was quickly weighed in air and then in tap water at 20 degrees Centigrade. The difference in the two weights in grammes recorded the volume of the specimen in cubic centimetres. The wet weight of the bone samples ranged from 6.93 to 16.21 grammes and their volume from 5.78 to 13.96 cubic centimetres.

The block of bone, after measurement of its volume, was then ashed in a silica crucible in a muffle furnace at 600 degrees Centigrade for twenty-four hours. The calcium content of the ash was estimated by standard chemical methods. The titration method of Vogel (1951) was used in most cases but later results were obtained with a flame photometer, at first in parallel with the chemical estimations, and then alone.

The assay is expressed as milligrams of calcium per cubic centimetre of bone.

RESULTS

The data in 100 cases are presented here. There were fifty-eight men and forty-two women whose ages ranged from eleven to eighty-eight years. Quantitative results are summarised in Tables I and II and in Figures 4 to 7. Data relating to individual cases are not given, but these were, of course, available for the statistical analyses to which we refer.

The average calcium content of the fourth lumbar vertebral body in the whole series was 68 milligrams Ca. per cubic centimetre volume of bone (range 38–102 milligrams per cubic centimetre). In 75 per cent of cases calcium values fell within the narrower range of 50–84 milligrams per cubic centimetre. Higher calcium contents were observed in younger persons. Thus, the two most heavily calcified vertebrae, with 102 and 101 milligrams Ca. per cubic centimetre respectively, were from men aged thirty-three and twenty-five years and one with 99 milligrams Ca. from a woman aged twenty-nine years.

Men tended to have more calcium in a unit volume of vertebral bone than women. The average in fifty-eight male cases was 72 milligrams Ca. per cubic centimetre (range 47–102) and in forty-two female cases the average was 64 milligrams Ca. per cubic centimetre (range 38–99). Even when allowance is made for the greater proportion of young men than of young women, as in any general hospital necropsy series, the lower calcium content of female bones is still evident at all ages (Table I).

Table I also indicates the decline of calcium in the vertebrae with increasing age, when the figures are expressed as averages. But a wide range of calcium values was still encountered in the older subjects, and with the number of results available it was not possible to calculate a statistically significant regression of calcium on age. A better correlation was achieved between age and radiographic density measured on a ten-degree scale.

Table II shows the average, and range of, calcium content of vertebrae manifesting
radiographic densities of four to ten step-wedge units. No bone was of less than four units density and none greater than ten. A relationship between calcium quantities and radiographic densities is apparent, as may be expected a priori, but we were concerned with the possibility of predicting the calcium content from the step-wedge reading, and of seeing whether in doing so it was necessary to take into account the factors of sex, age and number of days in bed, for which information was in each case available.

**TABLE I**

**Calcium Content (milligrams per cubic centimetre) of Male and Female Lumbar Vertebrae Bodies**

Expressed as Average Milligrams per Cubic Centimetre in Each Age Group

(Number of cases in each group in italics)

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>10-25</th>
<th>26-55</th>
<th>56-65</th>
<th>66-75</th>
<th>76-85</th>
<th>86-95</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>82 (8)</td>
<td>78 (13)</td>
<td>69 (18)</td>
<td>66 (15)</td>
<td>62 (4)</td>
<td>— (0)</td>
</tr>
<tr>
<td>Female</td>
<td>— (0)</td>
<td>70 (9)</td>
<td>44 (12)</td>
<td>63 (14)</td>
<td>59 (6)</td>
<td>48 (1)</td>
</tr>
</tbody>
</table>

**TABLE II**

**Calcium Content (milligrams per cubic centimetre) of Lumbar Vertebrae Bodies**

Grouped According to Their Radiographic Density in Step-wedge Units

<table>
<thead>
<tr>
<th>Radiographic density (step-wedge units)</th>
<th>Calcium (milligrams per cubic centimetre)</th>
<th>Total number of cases</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>Range</td>
<td>Number of cases</td>
<td>Number of cases</td>
</tr>
<tr>
<td>4</td>
<td>48</td>
<td>—</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>53</td>
<td>38-67</td>
<td>18</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>62</td>
<td>47-76</td>
<td>32</td>
<td>19</td>
</tr>
<tr>
<td>7</td>
<td>75</td>
<td>60-94</td>
<td>31</td>
<td>18</td>
</tr>
<tr>
<td>8</td>
<td>84</td>
<td>69-101</td>
<td>12</td>
<td>9</td>
</tr>
<tr>
<td>9</td>
<td>89</td>
<td>71-102</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>95</td>
<td>92-97</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>58</td>
<td>42</td>
<td></td>
</tr>
</tbody>
</table>

*Statistical analysis*—Miss Hilda M. Davies, of the Department of Statistics, kindly undertook the analyses and has reported to us as follows.

Multiple regression analyses of calcium on step-wedge reading, age and number of days in bed were carried out for males and females separately, and standard errors of regression coefficients were calculated. In both cases the regression of calcium on age and on days in bed proved to be non-significant: that of calcium on step-wedge reading was very highly significant, and therefore it can be assumed that some estimate of the amount of calcium can be obtained from the step-wedge reading and that in making this estimate there is no need to take either age or number of days in bed into account. This hypothesis was further supported when product-moment correlation and partial correlation coefficients were computed and it was found that the highly significant correlation between calcium and step-wedge reading of about 0.8, both for males and for females, was very little altered by correcting for either the age factor or the days in bed.

To investigate the possibility of a sex difference in such a prediction, a multivariate test of significance (Bartlett 1947) was applied to test the difference between the bivariate mean values of
Regression of calcium on step-wedge (males). Calcium (milligrams per cubic centimetre) plotted against radiographic density (step-wedge units) of lumbar vertebrae, showing regression curve and 95 per cent confidence limits (fifty-eight male cases).

Regression of calcium on step-wedge (forty-two female cases). As Figure 4.
Regression of calcium content on step-wedge units of radiographic density (males and females).

Regression of radiographic density on step-wedge units with age in years (males and females).
step-wedge reading and calcium for males and females. This gave an F criterion which was significant at the 5 per cent level, suggesting that there is a sex difference. An analysis of variance on calcium and step-wedge data for both sexes combined, testing the effects due to common regression, to difference in location of sex means and to difference in slopes of separate sex regressions, gave no significant difference between the separate slopes but gave an F criterion for difference in location of means which was significant at the 5 per cent level. This bears out the conclusions of the previous test.

Hence it appears that the regressions of calcium on step-wedge reading are parallel for the two sexes but slightly different in position. The separate regression lines of calcium on step-wedge reading for males and females were fitted by the usual method of least squares. The difference between the two regression coefficients was tested against its standard error by the usual Student's t-test and proved not significant, the value of t, in fact, being 0·56 with 96 degrees of freedom, which accorded well with the appropriate value of F in the analysis of variance and bore out the hypothesis that the data are samples from two populations which, though having a different mean value, have the same regression slope.

It seems, therefore, that the step-wedge reading can be used to give a prediction of the amount of calcium present provided that the sex is taken into account and the appropriate regression used. The 95 per cent confidence limits for such a prediction have been calculated in each case.

Figure 4 (males) and Figure 5 (females) show the distribution of calcium quantities in each step-wedge group, the regression of calcium on step-wedge readings and the calculated 95 per cent confidence limits for predicting calcium content from radiographic density in the step-wedge scale here used.

**TABLE III**

**Average Radiographic Density of Lumbar Vertebrae of Male and Female Subjects Arranged in Ten-year Age Groups**

<table>
<thead>
<tr>
<th>Age</th>
<th>Male</th>
<th></th>
<th></th>
<th>Female</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of cases</td>
<td>Average step-wedge reading</td>
<td>Number of cases</td>
<td>Average step-wedge reading</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-10</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11-20</td>
<td>5</td>
<td>8</td>
<td>—</td>
<td>—</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21-30</td>
<td>3</td>
<td>7.3</td>
<td>2</td>
<td>8.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>31-40</td>
<td>4</td>
<td>7.25</td>
<td>2</td>
<td>7.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>41-50</td>
<td>6</td>
<td>7.5</td>
<td>2</td>
<td>8.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>51-60</td>
<td>10</td>
<td>6.9</td>
<td>8</td>
<td>6.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>61-70</td>
<td>20</td>
<td>6.2</td>
<td>14</td>
<td>5.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>71-80</td>
<td>8</td>
<td>6.5</td>
<td>12</td>
<td>5.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>81-90</td>
<td>2</td>
<td>5</td>
<td>2</td>
<td>5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 6 compares the regression of calcium on density readings in the two sexes. The statistical analysis revealed that though the difference in slope of the two curves was not significant, the difference in the location of the means was. The implication of this result is not clear; the matter will be referred to in the discussion.

Table III shows the average step-wedge readings of vertebrae in ten-year age groups of men and women, and Figure 7 the regression of radiographic density on age. Miss Davies's statistical report was as follows.

It was noted that the negative product-moment correlations between step-wedge and age were significant and not much influenced by the presence or absence of the other effects, while that of calcium on age with step-wedge held constant was in both cases very small and well below the borderline of significance, implying some relationship between step-wedge reading and age but not
between calcium and age. In view of this an analysis of variance of similar type as before was carried out for the regression of step-wedge on age. This gave a very highly significant value for the combined regression \((F_{0.05} = 45.1)\) while there was no significant difference for the different sexes between either the separate slopes or the separate locations \((F\) slightly <1 in both cases), implying that there is no sex difference in the effect of age on step-wedge reading but that there is a marked overall regression effect.

The technique of radiographing parallel-sided sagittal slices of the vertebral bodies of uniform (1 centimetre) thickness laid flat on the film cassette enabled us to obtain very clear pictures of the trabecular structure, cortical thickness and contour of the bones. These features are well shown in Figures 2 and 3. In particular the highly rarefied bones of Case 88 in Figure 2 show how in osteoporosis the strong system of little bony plates of the normal vertebral spongiosa is reduced to a delicate web of thin struts, as Collins (1959) demonstrated in macerated specimens. Cortical atrophy is not an important feature of vertebral osteoporosis; the circumferential cortex of a vertebral body is thin in any case, and may become more prominent in osteoporosis—so-called stencilling of outline, by virtue of the greater translucency of the centrum. The bones illustrated in Figure 3 show an average radiographic density and texture. The radiograph of the bone slab gives much more information about cancellous texture than does the thin histological section (Figs. 8 and 9), where many trabeculae are incomplete and cut across obliquely, but such sections have been prepared in all cases in order to exclude osteomalacia, tumour infiltration or other causes of bone rarefaction or sclerosis.

Taking into account a qualitative assessment of the radiological and histological features of each vertebra we conclude that any vertebral slab having a step-wedge reading of five or less with this method shows unequivocal osteoporosis. It will be seen, therefore, from Table I that the incidence of osteoporosis of this degree in this series was 19 per cent. The youngest female to display spinal osteoporosis was aged fifty-one years and the youngest male fifty-nine years.

**DISCUSSION**

The methods that we have used in this investigation have been appropriate to the study of post-mortem material. They are less relevant to clinical studies, except in confirming that radiographic density of cancellous bone is statistically related to its calcium content (Table II and Fig. 4), a fact that was implicit in Virtama’s (1957) radiological investigation of the phalanges post-mortem and was to be expected on a priori grounds. By using parallel-sided bone slabs of standard thickness, devoid of superimposed soft tissues, the radiographic measurement by means of a step-wedge and a photo-electric cell becomes much simplified. To counteract the variables of soft-tissue shadows and distance between bone and film in clinical radiography, complex methods must be used, such as those devised by Mack, Brown and Trapp (1949). For our present purposes we are satisfied that the simple radiographic procedures, as outlined in the description of methods and in Figure 1, give a sufficiently accurate prediction of the mineral content of the bone specimens to be used as a method in surveying post-mortem material that is much quicker than chemical analysis. In individual cases some discrepancies between radiological and chemical measurements were encountered (Figs. 5 and 6), and the peculiar fact emerged in statistical analysis that, though falling into the same step-wedge group of density, female bones tended to have slightly lesser amounts of calcium than those of males (Fig. 6). The reason for these discrepancies probably lies in the fact that in a specimen of cancellous bone we are radiographing a system of calcified trabeculae dispersed to a varying degree, depending on the porosity or mesh of the bony network, in soft marrow tissues that also absorb some rays. Mack, Brown and Trapp (1949) stated as a generalisation that calcium is responsible for about 80 per cent of x-ray absorption by bone, but this proportion obviously varies with the ratio of calcified structure to soft-tissue mass.
Fig. 8
Histological section of lumbar vertebral body (celloidin-paraffin embedded, stained with haematoxylin and eosin). This bone, of low normal density, showed a radiographic reading of six step-wedge units and a calcium content of 72 milligrams per cubic centimetre.

Fig. 9
Histological section, as Figure 8, of vertebra of high normal density showing a radiographic reading of nine step-wedge units and a calcium content of 90 milligrams per cubic centimetre.
The radiographic scale based on a ten-step aluminium wedge gave in effect only seven degrees of bone density, since no bone was encountered so rarefied as to match with the first three steps of the wedge. Ninety-three of the 100 cases showed densities corresponding with the four step-wedge units five to eight (Table II). The six bones of greater density showed no pathological variation of either radiographic texture (Fig. 2) or of histological structure. The solitary example of a bone showing a step-wedge reading of only four was from an old lady of eighty-eight who on account of pemphigoid had been treated for several weeks before her death with ACTH and prednisolone, which may have accentuated the osteoporotic process. In no other case did we learn of diseases or treatments or other circumstances that might have caused or accentuated osteoporosis.

Radiographs of the bone slabs give a very clear picture of the cancellum of the vertebral bones (Figs. 2 and 3). They also show to what a small extent cortical compact bone enters into the specimen taken in this manner. Indeed, both radiographic and chemical results mainly measure variations in the size, number and degree of separation one from another of trabeculae and differences in the degree of calcification of the osseous rami. It is highly probable that the osseous rami do differ in the extent to which they are calcified. Though osteoporotic bone has not been specially investigated, Amprino and Engström (1952) showed by microradiography not only that the osteones of cortical bone vary in their degree of mineralisation, but that more imperfectly calcified structures are present in spongy than in compact bone. These structures with a low content of mineral salts represent bone in the process of being mineralised (Engström 1956). It seems important, therefore, with a view to establishing the direction of metabolic activity of osteoporotic bone, that the vertebral cancellum should sometime be studied either by microradiography or by interference microscopy (Davies and Engström 1954). In every case, as in the present series, ordinary histological preparations must be examined, because this is the only way of excluding gross variations in the calcium/osteoid relationship, such as pertains in osteomalacia, and of recognising hyperparathyroidism or other pathological causes of bone resorption. In eight of the 100 cases the radiograph revealed a patchy unevenness of the shadow of the lumbar vertebral body. This was unrelated to kyphosis, other deformity or apparent disease. It was not explained, but it did not interfere with our obtaining an average photometric reading of density for the whole bone.

Loss of the transverse trabecular markings and accentuation of the vertical markings is a valuable and constant sign of osteoporosis (Fig. 2). This characteristic was found to correspond with a density reading of five step-wedge units or less, and we are confident in recording as osteoporotic the nineteen cases (eight male, eleven female) showing this degree of rarefaction. The other well known sign of osteoporosis in clinical radiology—that of biconcavity of the vertebral body, the so-called fish vertebra—was in our experience of little value in assessing osteoporosis. It was easy to measure the radiographic profile of the vertebral body and to calculate the ratio of least to greatest vertical depth, as Barnett and Nordin (1960) suggested. Vertebrae in six cases showed this ratio to be less than 75 per cent, a considerable degree of biconcavity; in three the vertebrae were judged to be osteoporotic by other standards, but in the other three cases the vertebrae were of normal density and calcium content. As Collins (1949, 1959) has pointed out, biconcavity results from the pressure of the still turgid intervertebral disc, and if osteoporosis develops after the discs have degenerated or collapsed the vertebral bodies will not become so deformed. Biconcavity, in short, is a reflection of mutual stresses between the vertebral body and its adjoining discs; it is not a function of changes in the bone alone. If biconcavity is conclusive evidence of deformation of a softened vertebral body, then our finding of some biconcave vertebrae of normal density and calcium content can only be explained by assuming that these bones have become remineralised after a period of osteoporosis. But this matter needs further investigation.
The chemical results giving the amount in milligrams of calcium per unit volume (1 cubic centimetre) of anatomical bone are of special interest because, so far as we know, calcium assays have not previously been made of human vertebra, and this method of expressing the results has not been generally adopted in other bone analyses, although it seems to us to be the only way in which the results can be expressed in order to measure what we call osteoporosis and to tally with readings of radiographic density. The range of calcium contents encountered was quite surprisingly wide, from as little as 38 to as much as 102 milligrams Ca. per cubic centimetre of bone, but 75 per cent of cases fell within the narrower range of 50–84 milligrams per cubic centimetre. The average for this series was 72 milligrams per cubic centimetre for men and 64 milligrams per cubic centimetre for women. These figures cannot be taken as averages of general application: much depends on the ages of the subjects comprised in any sample, since it is clear that there is a steady decline with age both of calcium content (Table I) and of radiographic density (Table III and Fig. 7). Even in age groups the range of calcium results is still wide, and the occasional old person may be found with vertebrae that are highly calcified though histologically normal. It was not possible for us to establish a level of calcium content below which a vertebra may be held to be osteoporotic. It was more practicable to name a bone as osteoporotic on radiographic density and texture, other causes of rarefaction being excluded by histological examination. Nevertheless, although there is some overlap of individual results, the generally low calcium of the osteoporotic bones of step-wedge values four or five is apparent in Table II. The average calcium for the eight osteoporotic male cases was 56 milligrams per cubic centimetre as compared with an average of 74 for the fifty male cases judged to show no osteoporosis. In the eleven osteoporotic female cases the average calcium was 50 milligrams per cubic centimetre as compared with an average of 69 for the remaining thirty-one female cases judged to show no osteoporosis.

When the results of calcium analyses of our series of vertebrae are plotted the curve is a smooth one for either sex, though the peak for females is to the left of the male peak (Fig. 10). The skewness of the curves may well be due to the inclusion in our series of a majority of older subjects who tend to show lower calcium values. The main lesson to be learnt from a study of these curves, and of the distribution of the density readings shown in Table II, is that there is no separate peak which might indicate that the sample contained two populations of "normal" and "pathological" bones.

The results indicate the numerical range of mineral disturbances to which the vertebrae are subject. In round figures we may state that the lumbar vertebrae among a mainly elderly hospital population are calcified to the average extent of 70 milligrams Ca. per cubic centimetre, with extreme divergences of 30 milligrams on either side, bones containing 40 milligrams Ca. per cubic centimetre being severely osteoporotic, and bones containing 100 milligrams Ca. per cubic centimetre being mainly among the strong bones of young men. Statements have often been made that a bone must lose at least half its calcified bone mass before it can be recognised as osteoporotic in the clinical radiograph (Fraser 1959). Fusil (1953), who largely excluded the soft-tissue interference by using vertebral body slabs of similar thickness to our own, reported evidence of radiological demineralisation when only 3 per cent of the original calcium content was extracted, and with an extraction of 30 per cent radiological changes were strongly evident: but when the body soft tissues were interposed the extraction of 60 per cent of the calcium was necessary in order to show a notable abnormality. Our analyses partly confirm these findings since, on the average, vertebrae judged to be osteoporotic contained 24 per cent less calcium in the male and 28 per cent less in the female than vertebrae held to be normal. There is, of course, no way of estimating the loss of calcium in any individual bone affected by osteoporosis, because its original calcium content remains unknown. Indeed, it is likely that the vertebrae in any individual are most heavily calcified in youth and then proceed to lose calcium (and bone substance) progressively throughout life. It seems that, in osteoporosis, we may be confronted once again, as in osteoarthritis (Collins 1949) and,
perhaps, as in hypertension (Pickering 1955), with the problem of determining how and in what manner changes that are the almost universal accompaniment of age are to be distinguished as pathological.

Our present study adds little to our knowledge of the incidence and etiology of osteoporosis. All studies of incidence are based on the somewhat arbitrary and self-imposed standards of the investigators. By our own standards we have called osteoporotic the lumbar vertebrae of eight out of fifty-eight males (14 per cent) and eleven out of forty-two females (26 per cent) in a random series of hospital necropsies. Using entirely different standards, though equally arbitrary—namely, the flotation of cubes cut from the vertebral centra in copper sulphate solution of specific gravity 1.050—Collins (1959) studied a similar but larger series of hospital necropsies and calculated the incidence of spinal osteoporosis as 8 per cent among 189 men and 18 per cent among 147 women aged forty years or more. As in our present series, only one case judged to be porotic was encountered below the age of sixty years.

Cooke (1955) reported that osteoporosis is six times commoner in women than in men, but Jackson (1958) found twenty-seven male to eleven female patients with osteoporosis of unknown cause developing in men before the age of fifty-five and in women before the menopause. We have no information of the age at which porosis first developed in our cases but only one patient, a woman aged fifty-one, was younger than sixty-one years at the time of death. The period of confinement to bed during the last illness was noted, but could not be correlated with the state of the vertebrae. The nature of the fatal illness was too varied in this small series of cases to have any apparent bearing on the condition of the bones.

**SUMMARY AND CONCLUSIONS**

1. Radiological, chemical and histological examinations have been made of the lumbar vertebral bodies in 100 necropsies on patients dying in a general hospital, with a view to determining the range of variation of calcium content and radiographic density in normal and osteoporotic bone.
2. Radiographs were made of sagittal mid-line vertebral body slabs uniformly one centimetre in thickness, and the radiographic density of these specimens was measured in relation to an aluminium step-wedge of one to ten units. Radio-opacity of different vertebrae ranged from four to ten units. The specimen radiographs also clearly revealed the trabecular structure and the lateral profile of the bones.

3. Calcium was chemically estimated and expressed as weight of the element per unit volume of the whole bone mass (that is, of anatomical bone including soft marrow tissue). It ranged from 38 to 102 milligrams per cubic centimetre of bone. In 75 per cent of the cases the range was 50–84 milligrams per cubic centimetre. High calcium values were mostly encountered in young adults, and the calcium per unit volume tended to diminish with age; but a wide range of calcium was still encountered in the older subjects and a better correlation with age was achieved by radiographic density. Both calcium content and radiographic density tended to be higher in the male than in the female bones at all ages.

4. The results of both calcium and radiographic density showed a smooth distribution curve, though skewed through the inclusion in the series of more older people with less mineralised bones; the absence of a double peak in these curves suggests that the examinations were made on a homogeneous population and does not indicate a separate pathological group of osteoporotic subjects.

5. Arbitrary standards must be used to distinguish osteoporotic from normal bones, since neither radiological measurement or chemical assay, nor histological assessment, reveals a point at which the two groups can be separated. In the present series it seemed to us satisfactory to regard as abnormal all bones showing a radiographic density of five or less step-wedge units, and by this standard nineteen of the 100 cases (eight male, eleven female) were deemed to be osteoporotic. Histological examination excluded other forms of bone rarefaction.

6. The regression of calcium on the density measurements proved to be statistically significant and was not affected either by age or by the number of days in bed during the last illness. A small difference between the sexes was apparent, there being slightly less calcium in female than in male bones of equal radiographic density. Provided this is taken into account, the radiographic density scale can be used to predict the calcium content of vertebral bone specimens and should prove a rapid and accurate method in a survey of osteoporosis in post-mortem room material.

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REFERENCES


ASSESSMENT OF VERTEBRAL OSTEOPOROSIS BY RADIOGRAPHIC AND CHEMICAL METHODS


