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Three-dimensional photographic study of cancellous bone in human fourth lumbar vertebral bodies

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Abstract. In an analysis of the 3D architecture of cancellous bone, two-dimensional techniques are of limited value. A simple technique employing stereophotographs of whole sections of lumbar vertebrate made possible a detailed description of the 3D structure of the normal fourth lumbar vertebral body and its changes with ageing and osteoporosis. Parallax measurements were used to calculate the real lengths of horizontal trabeculae. The bone presented a continuous spectrum of microstructure, from a honeycomb of tubes, to plates and braces and, finally, fragile rods. A distinct pattern was produced in osteoporotic samples by the removal of horizontal and selected vertical trabeculae followed by a thickening of the remaining vertical trabeculae in the peripheral regions. Very long, thin horizontal trabeculae were formed in all three zones (superior, middle and inferior) during this process. The observation of porotic architecture in intact specimens points to the inadequacy of the clinical criterion of the occurrence of a fracture in judging the osteoporotic state.

Key words: Bone – Cancellous – Trabecular – Age – Osteoporosis

Introduction

Age-related loss of vertebral cancellous bone leads to spinal osteoporosis and crush fractures (Lindahl and Lindgren 1962; Arnold et al. 1966; Atkinson 1967; Dunnill et al. 1967; Arnold 1973; Cann et al. 1985; Mosekilde et al. 1987). However, the loss of strength of vertebrae is proportionately greater than loss of osseous tissue (Bell et al. 1967), because the biomechanical competence of cancellous bone is dependent not only on the absolute amount of bone present, but also on the cancellous microstructure (Galante et al. 1970; Pugh et al. 1973; Kleerekoper et al. 1985; Mosekilde et al. 1987; Jensen et al. 1990).

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Two-dimensional "histomorphometric" descriptions of cancellous bone architecture in vertebral bodies and iliac crest biopsies have included measurements such as mean trabecular width, area, trabecular separation and interconnectedness (Wakamatsu and Sissons 1969; Garrahan et al. 1986; Aaron et al. 1987; Bergot et al. 1987, 1988; Compston et al. 1987; Birkenhager-Frenkel et al. 1988; Bergot et al. 1988; Cosman et al. 1992) and trabecular bone pattern factor (Hahn et al. 1992).

Vesterby et al. (1989) described a stereological parameter for bone structure (the star volume) which provides an index of the connectedness of the marrow space: star volume increases with age in both lumbar vertebrae and iliac crest (Vesterby 1990). A medium-resolution computed tomographic technique for the direct examination of 3 D bone structure in vitro was described by Feldkamp et al. (1989). A correct procedure for estimating connectivity, which must involve the reconstruction of the entire bone, has been given by Odgaard and Gundersen (1993).

Key contributions describing the architecture of the cancellous bone of the vertebral bodies were made by Arnold (1968, 1970), Amstutz and Sissons (1969), Whitehouse et al. (1971), Schmorl and Junghans (1975), Singh (1978), and Parfitt et al. (1983). Arnold (1968, 1970) studied the macroscopic structure of vertebral cancellous bone using 2- to 3-mm-thick sagittal and horizontal sections of vertebral bodies. He described regional variations of cancellous bone in normal sections, and changes that occur during the process of ageing. Parfitt et al. (1983) demonstrated that age-related bone loss occurs principally by a process that removes entire structural elements of bone. He proposed that the process of removal is initiated by perforation of trabecular plates: progressive enlargement of perforations leads to the conversion of plates to rods.

Delling and co-workers investigated sagittal sections of whole spines, using techniques which permitted a combined two- and three-dimensional histomorphometrical analysis (Delling 1989; Hahn et al. 1989, 1992; Pompesius-Kempa et al. 1989; Vogel et al. 1989). While confirming previous reports of the role of perforations and loss of horizontal trabeculae during age-related bone loss, they found that the bone volume depends heavily on the number of plates, whereas there is no correlation, or even an inverse relation, between bone volume and the number of rods. Using an index of intertrabecular connectedness (trabecular bone pattern factor: TBPf) they reported a correlation between bone volume and trabecular connectedness.

Vertebrae develop by endochondral ossification of cartilaginous models. It is generally believed that bony elements are laid down around invading blood vessels: these elements are later remodelled, depending on the functional need and the mechanical forces that they are subjected to, into those seen in the adult vertebrae. It is not clear to what extent these bony elements that first develop are maintained into adult life, and how far they influence the adult structure. Vascularization of the vertebrae changes with age (Ratcliffe 1982, 1986).

In illustrating the structural changes in cancellous bone, it is easy to provide images at a relatively high magnification showing a very small amount of bone tissue taken out of context. One small field of view might show a small amount of bone tissue per unit volume and normal continuity of structure in an otherwise obviously osteoporotic individual. Another might show a rather open structure in small regions of an otherwise dense cancellous bone structure. Hence there is a need for an imaging technique which is unprejudiced by tissue sampling and selection.

The findings we report here are part of a larger study in which we explored several different methods, scanning electron microscopy (SEM) included, to provide a broad view of the changes which can best be assessed in detail by SEM methods. Although SEM has substantial advantages in resolution and depth of field over conventional light optical methods, it has disadvantages. Conventional SEM specimens have to be dry and pumped down to a high vacuum and usually have to have a continuous conductive coat applied to them, though the aim of the prevention of charging is often not achieved in practice (Whitehouse et al. 1971; Mosekilde 1990). In addition, most SEMs will not provide a tolerably distortion-free image of the entire field of view of an intact section through an adult human lumbar vertebral body. In the present article, we consider the use of a simple optical means to provide a contextual image of the entire bone sample.

Materials and methods

The fourth lumbar vertebral body was removed at post mortem from 36 adult subjects (18 male, 18 female, age range 30–91 years). Three female subjects and one male had suffered fractures and were confirmed osteoporotics. Samples were defleshed and stored immediately in 70% ethanol.

Plane parallel sections 4 mm in thickness were cut using a water-cooled low speed diamond saw (Buehler Isomet). For sawing, the lumbar vertebral bodies were clamped by means of a device with several screws which held the bone firmly in regions well away from the regions to be sectioned, without providing a crushing force to the bone as a whole. A local penetration was used to grip the cortex of the bone. The regime did not break trabeculae, but cut through them. Sections were cut in the median (IJ) plane (i.e. that of the diagram in Fig. 1) and the para-sagittal plane, with some horizontal (AB, CD and EF in Fig. 1) and coronal (GH in Fig. 1) sections for comparison.

To remove marrow and soft tissues, the 4-mm sections were treated at 37° C with 2% hydrogen peroxide (15 times dilution of fresh stock concentrate) for 24 h, cleaned with a jet of water, defatted for a few hours in chloroform/methanol, and air dried.

When examined under a stereo-binocular microscope, the 3D structure of these samples could be clearly discerned, but only a small area of bone was in the field of view, and the depth of focus was insufficient. The following procedure was therefore used to produce stereo-macro-photographs.

Samples were mounted on an adjustable tilting stage with the tilt axis in the mid-line of the sample, then, with an 80-mm macro lens for an Olympus OM2 35-mm camera, stereophotographs were produced with a preset tilt angle difference of 10° between pairs. A very small aperture (F22) was used to increase the depth of field. Prints of 165 mm × 115 mm were examined under a Hilger and Watts SB180 mirror stereoscope with parallax measuring facility (Martin 1966). The 3D structure of the whole section could be viewed and the whole thickness of the section was in focus. In addition to the observation of 3D structure of trabecular bone in different regions, different groups of trabeculae (e.g. vertical and horizontal) could be identified and counted at a convenient scale. Vertical trabeculae were counted from the anterior to posterior border at superior, middle and inferior levels (AB, CD, EF Fig. 1). Horizontal trabeculae were counted in the coronal plane (GH Fig. 1). The biconcavity indices of the vertebral bodies used during the study were also calculated by dividing the mid-vertical height of each section by its anterior vertical height.

The relationship between height difference and parallax could be simply found by measuring the parallax for features at the front and at the back of sections of measured thickness. Height differences between pairs of points in the two photographs could be determined from their parallaxes, and, combined with measurements of X,Y co-ordinates, the real size of any elements could be calculated. The lengths of unobscured horizontal trabeculae were determined in the superior, middle, and inferior regions of selected young, old and osteoporotic samples, irrespective of their orientation in the horizontal plane.

The rotation of the component images of the stereo-pairs is determined by the requirements for stereo-viewing in each case and not by any standard anatomical convention. Millimetric grids indicate magnification

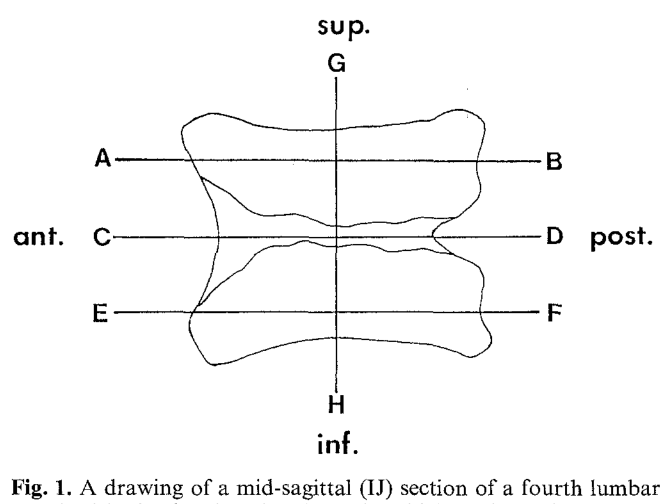
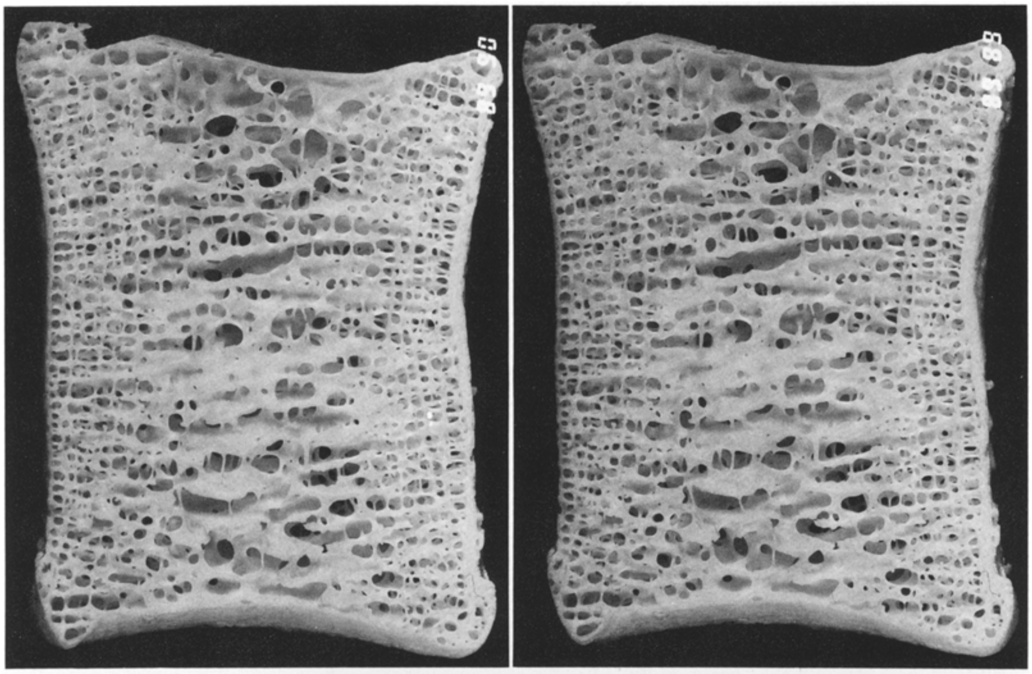


Fig. 1. A drawing of a mid-sagittal (IJ) section of a fourth lumbar vertebral body showing the planes in which horizontal (*AB, CD, EF*) and coronal (*GH*) slices were prepared



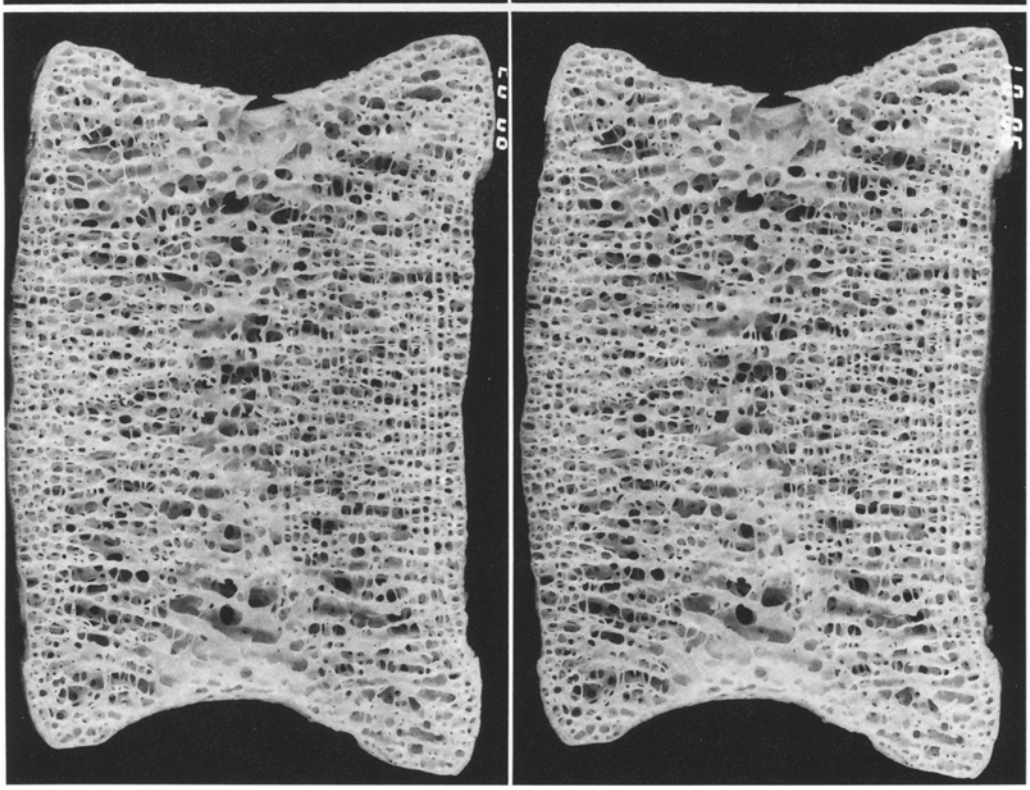


Fig. 2. Preparation of fourth lumbar vertebral body from 31-year-old male subject; mid-sagittal (median, IJ) section, superior to left

Fig. 3. Coronal (GH) section through midpoint of the vertebral body of 30-year-old female subject; superior to left

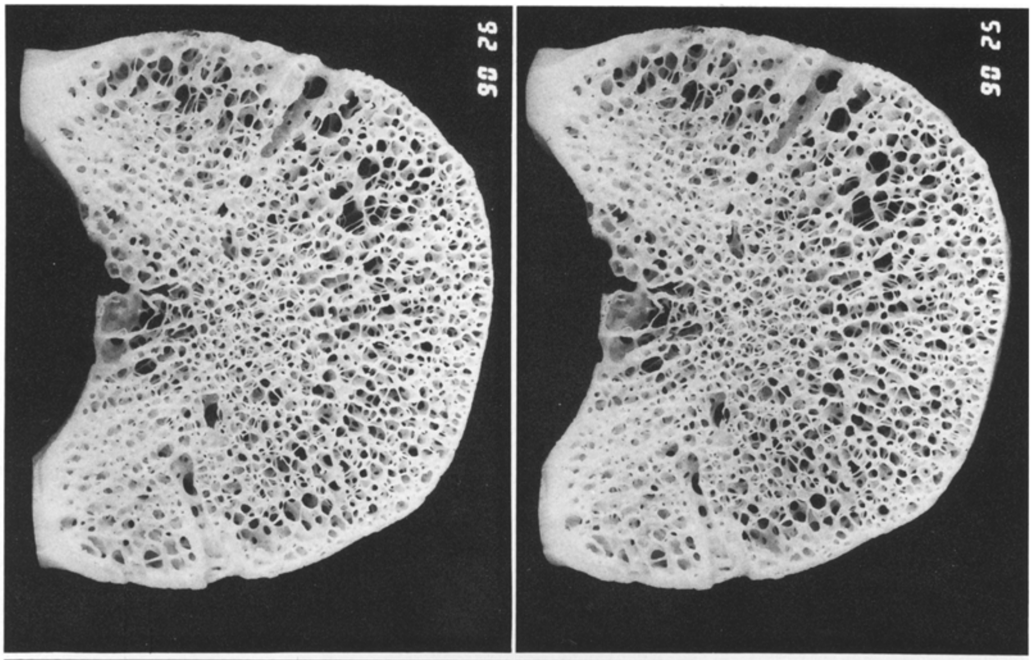
Results

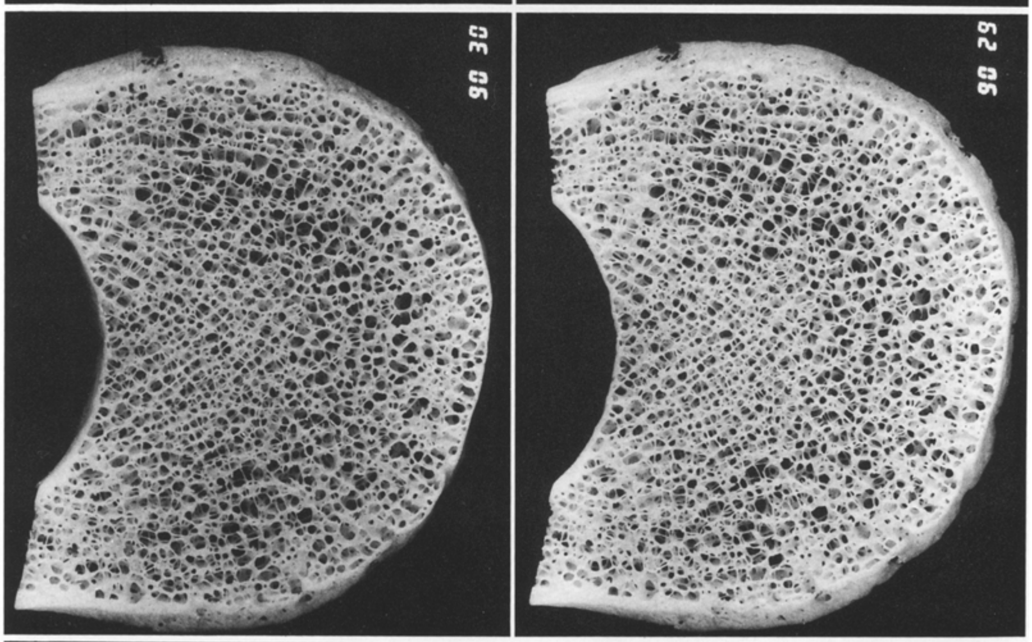
General structure

Most of the bone is organized as a honeycomb of continuous plates which surround mainly tubular (soft-tissue-filled) spaces that are oriented predominantly in the longitudinal axis of the bone.

The spaces are smaller in the upper and lower thirds of the body than they are in the middle third. They have some continuity with other spaces in all directions, but there is little patent transparency in a cleaned, macerated 4-mm section of young mature adult bone viewed in any vertical plane, either sagittal (IJ) or coronal GH (Figs. 2, 3, 8, 9). In contrast, horizontal (transverse) sections (AB, CD, EF in Fig. 1) at any level show a great deal of openness in a 4-mm section thickness, indicating that the tubular spaces of the honeycomb run for distances greater than 4 mm.

The sizes of the space in the honeycomb and the apparent continuity of the space differ at the different lev-





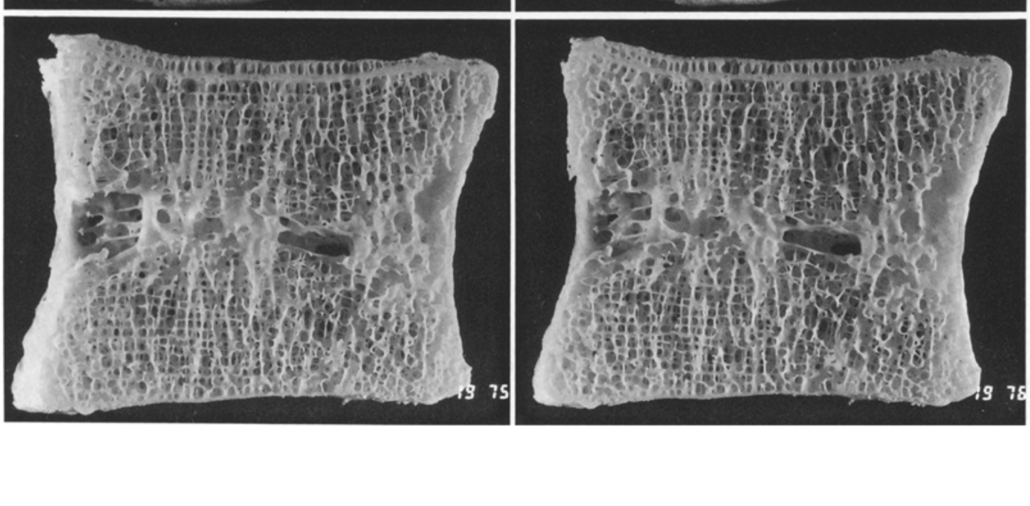
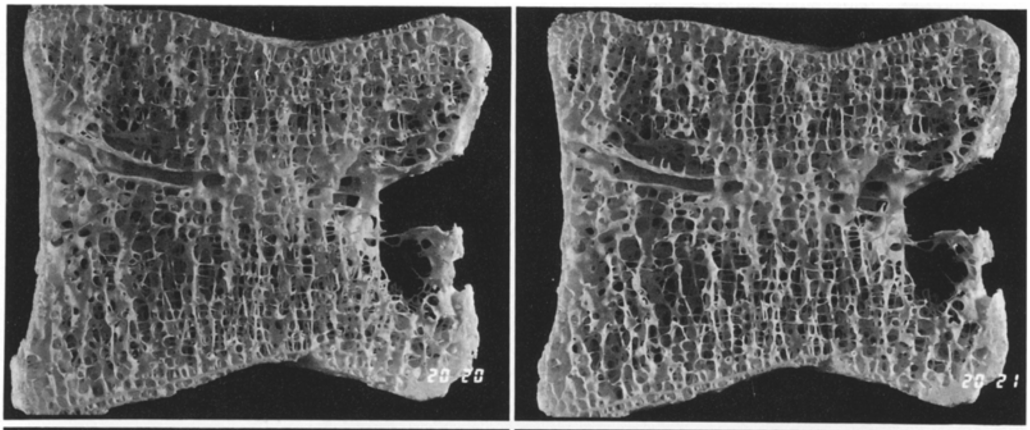
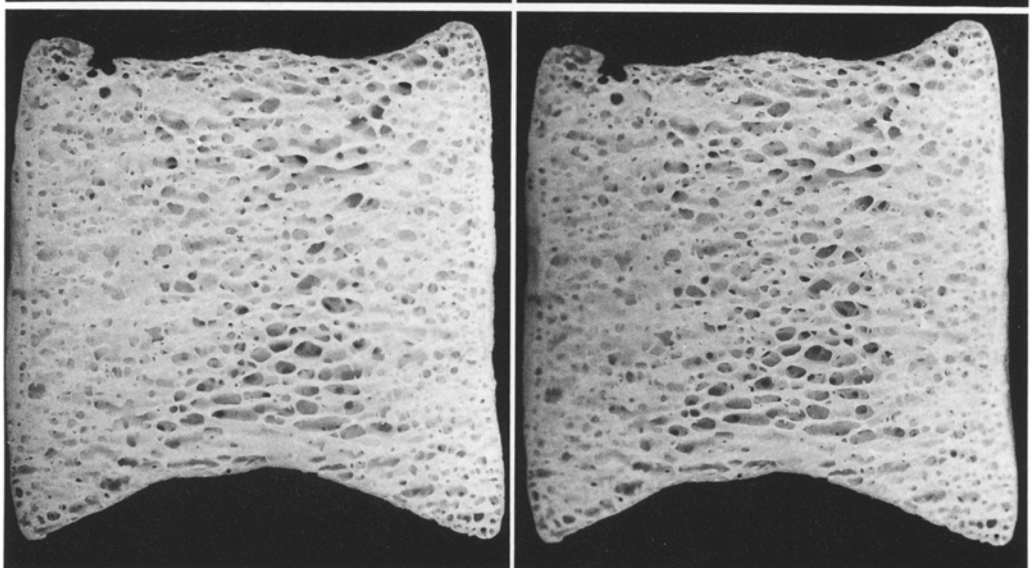


Fig. 4. Horizontal (CD) section through the middle zone, vertebral body of 30-year-old female subject. Note two canals for blood vessels directed centrally and posteriorly from lateral walls; posterior to left

Fig. 5. Horizontal (AB) section through the superior zone of the same vertebral body as Fig. 4; posterior to left

Fig. 6. Mid-sagittal (median, IJ) section of vertebral body of 50-year-old male subject. Note that double vertebral end plates are present at the superior end of the section; posterior to right





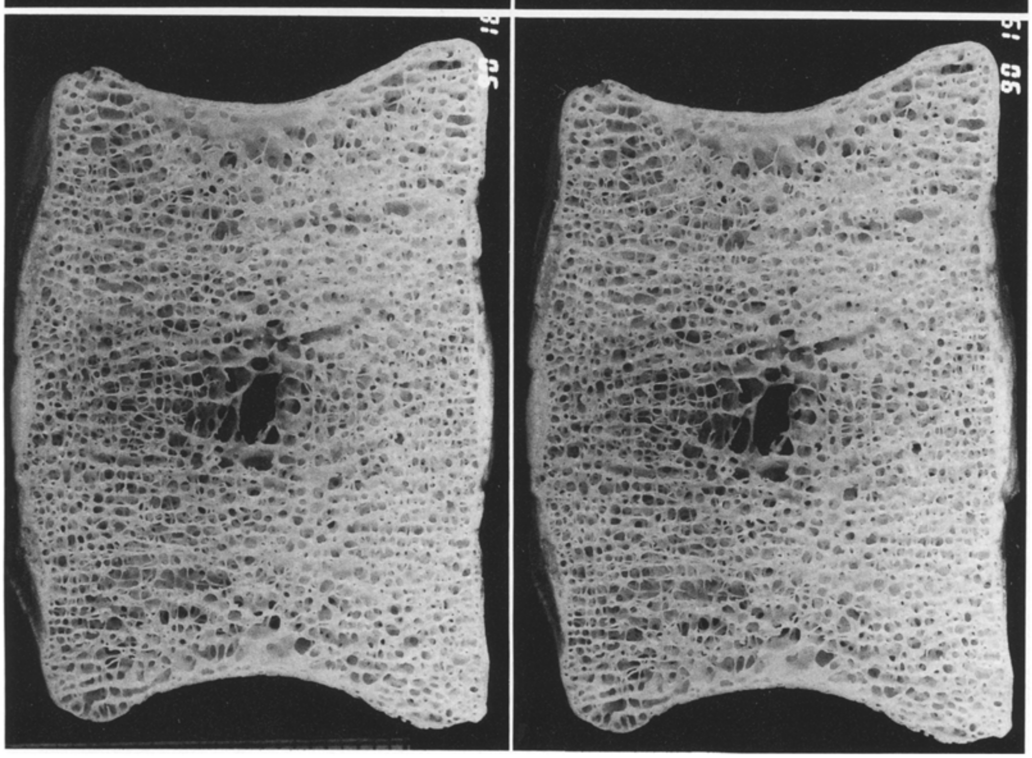
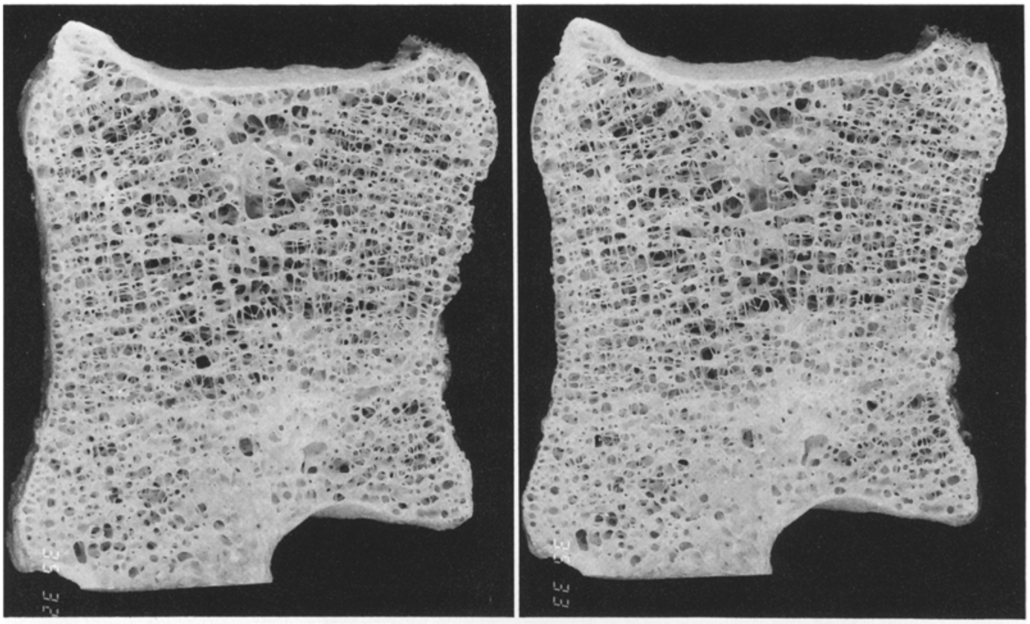
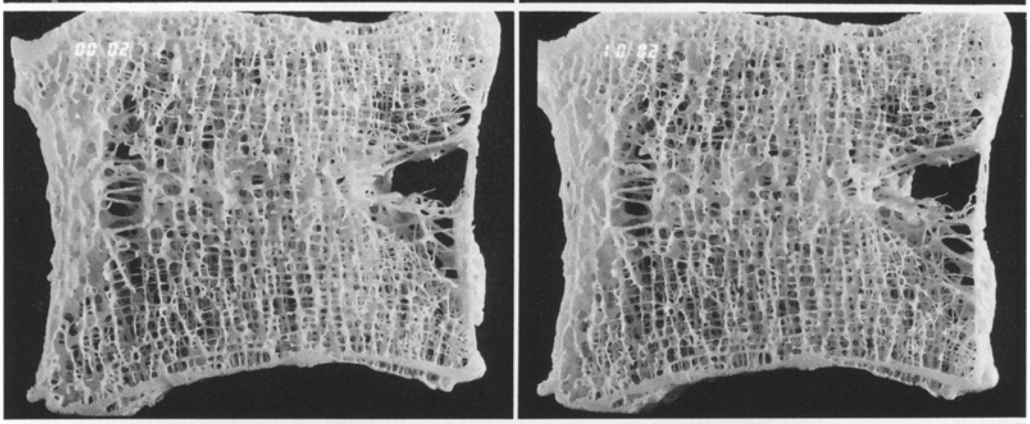


Fig. 7. Mid-sagittal (median, IJ) section through vertebral body of 64-year-old male subject. The deficiency at the posterior side represents the point of entry of basivertebral vessels. A canal for a large vessel can also be seen traversing the middle zone; posterior to right

Fig. 8. Coronal (GH) section through the most anterior part of the vertebral body of 30-year-old female subject; superior to left

Fig. 9. Coronal (GH) section through the posteriormost part of the vertebral body of 30-year-old female subject. The central hole bounded by plates represents the portal of entry of the basivertebral vessels; superior to left





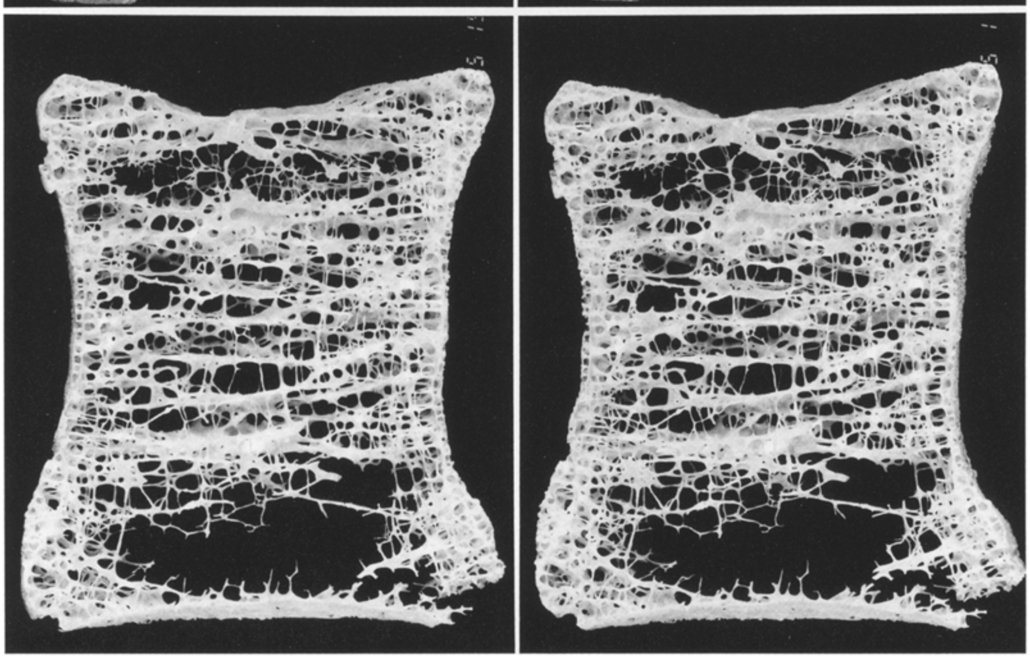
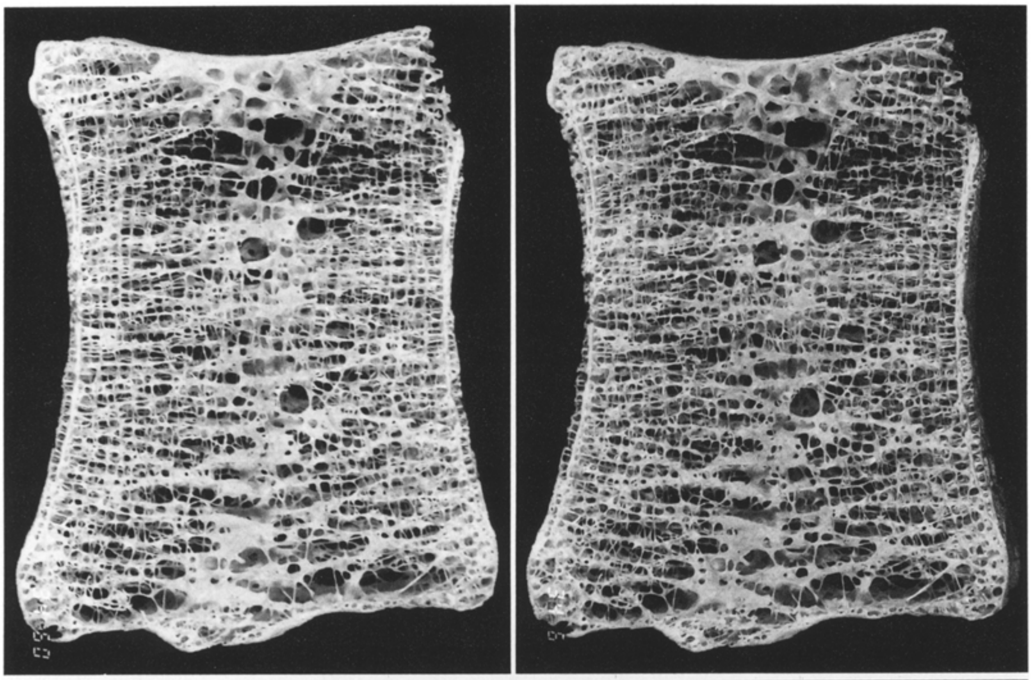


Fig. 10. Parasagittal (parallel to IJ) section through vertebral body of 69-year-old male subject; superior to left, anterior top

Fig. 11. Mid-sagittal (median, IJ) section through vertebral body of 71-year-old male subject; anterior to left

Fig. 12. Mid-sagittal (median, IJ) section of vertebral body of 89-year-old osteoporotic female subject with complete loss of normal architectural pattern. The break at one corner of the section has occurred during sample preparation



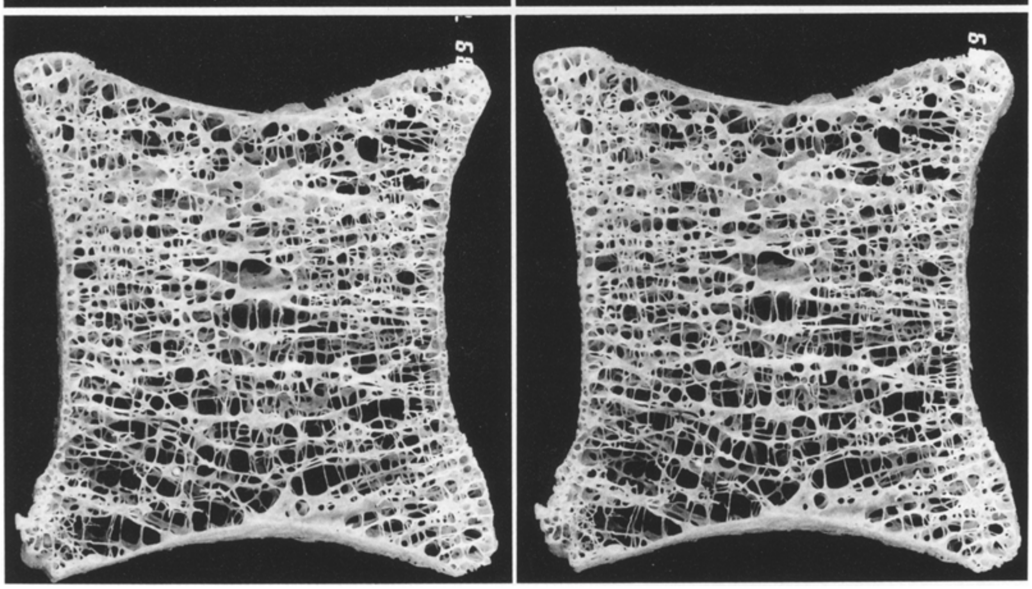


Fig. 13. Mid-sagittal (median, IJ) section of vertebral body of 89-year-old male subject

Fig. 14. Mid-sagittal (median, IJ) section of vertebral body of 88-year-old female subject with osteoporotic architecture

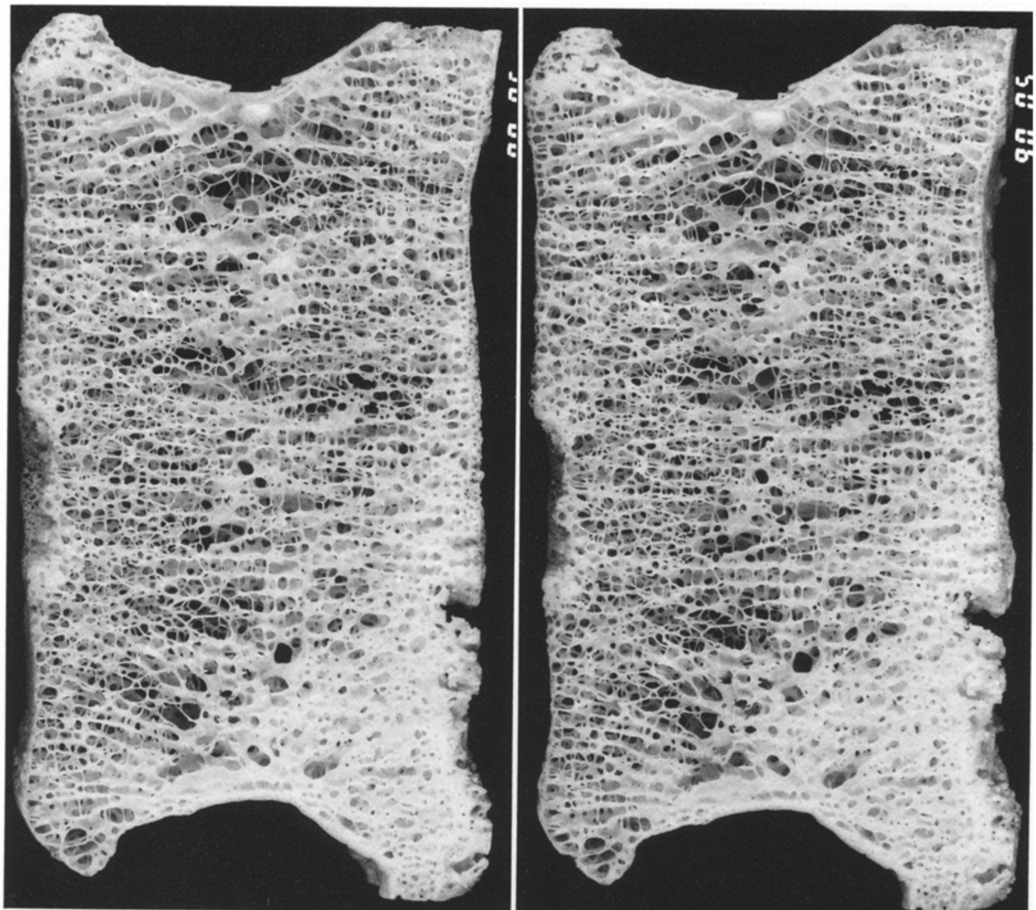
els in the bone. In particular, there are more horizontal elements (which relate to blood vessels) in the central third of the bone (Figs. 4, 6, 7). Large apparent vacancies in the sections relate to the outside form of the bone.

In elderly and obviously osteoporotic material (Fig. 12), large spaces may exist within the vertebral body where there is no bone. In individuals with substantial porosity, the remaining vertical elements are plates, forming the walls of large tubular spaces that run mainly in the longitudinal axis of the bone. The remaining bone tissue is reduced to thin rod-like elements running predominantly in the horizontal axis of the bone. In elderly individuals who have retained much of the numerical density of trabecular elements, the same tendency to more bulky longitudinal (vertical) elements and more slender horizontal (transverse) elements can still be identified (Figs. 13, 14, 15).

Transverse (horizontal) sections of porous elderly bone (Figs. 16, 17) reveal that the elements seen as plates in longitudinal section views are extremely thin in cross section. Figures 16 and 17 show both the elements which will be seen as plates and thin transverse (horizontal) struts in the longitudinal (vertical) section. Frankly osteoporotic samples sometimes showed small globular features on some struts; these are the sites of microfractures repaired by microcallus (Fig. 19).

Detailed description of morphological findings

The typical, normal structure of the mature adult vertebral body can be seen in Fig. 2. The cancellous structure can be divided into three zones. The middle zone consists of relatively larger plates or laminae oriented mainly



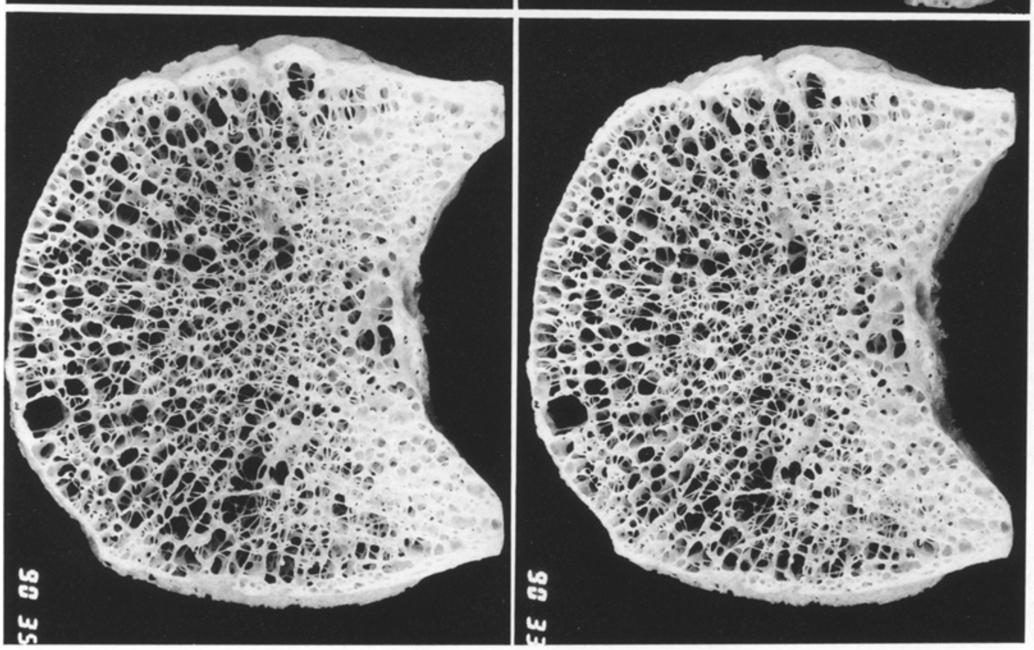


Fig. 15. Coronal (GH) section through the middle of the vertebral body of an 88-year-old female subject

Fig. 16. Mid-horizontal (CD) section through vertebral body of 88-year-old female subject

in the vertical plane, whereas the upper and lower zones have shorter elements apparently arranged as much transversely (horizontally) as vertically; 3D views show that the plates of the middle zone are the common walls of vertical tubes.

In a roughly triangular area representing the most anterior region of the middle zone, some samples show such extensive chasms, walled by plates, that the exact 3D arrangement cannot be so simply described (Figs. 2, 6). Others clearly show horizontal tubes of larger (Fig. 7) and smaller diameter. The structure can be better studied in the coronal plane (GH Fig. 8), in which the contrasting architecture of the superior and inferior zones compared with the middle zone is also

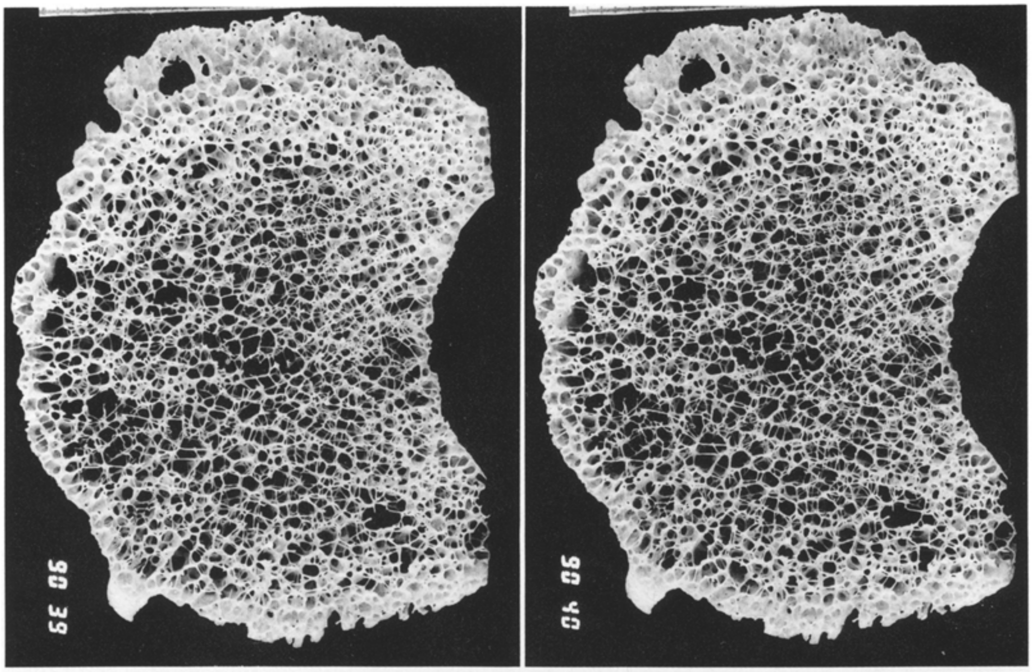


Fig. 17. Horizontal (AB) section through the superior zone of the vertebral body in Fig. 16. The irregular outline of the section is due to the formation of osteophytes. Several microcallus patches are present in this section

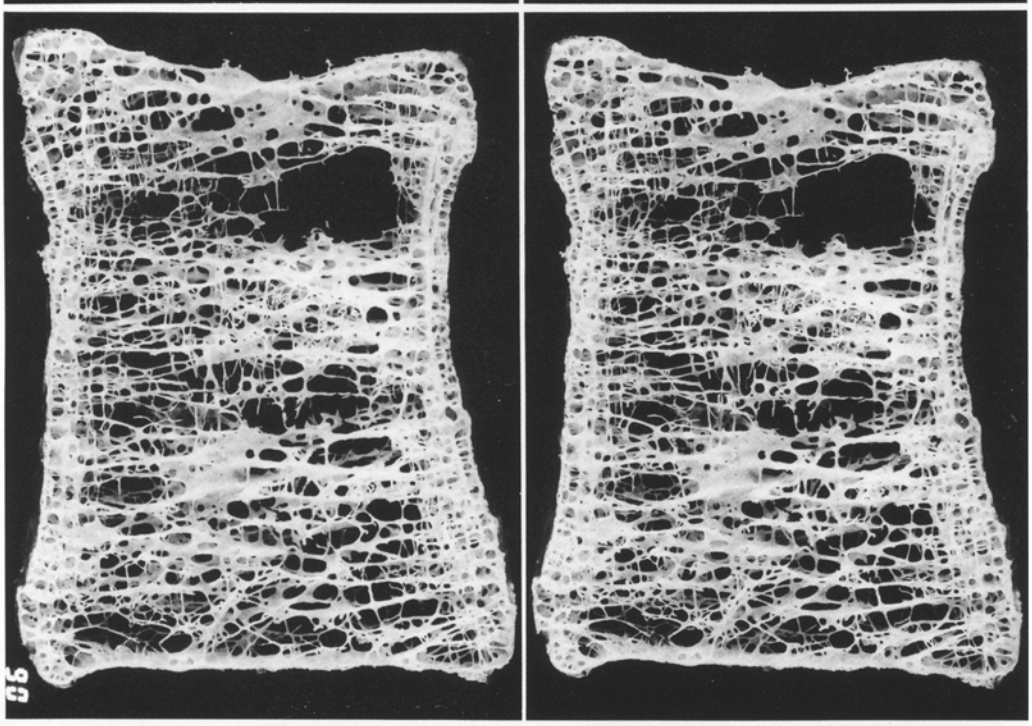


Fig. 18. Mid-sagittal (median, IJ) section through vertebral body of 89-year-old osteoporotic male subject

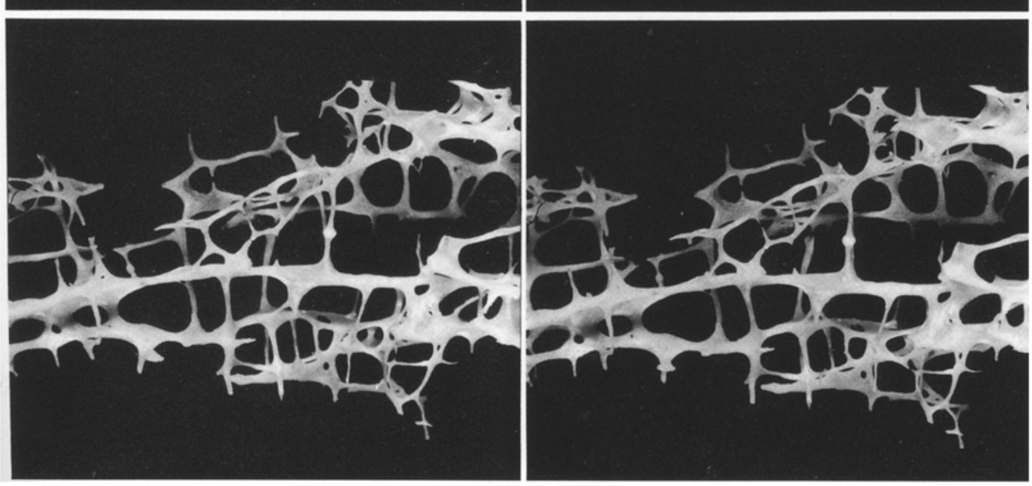


Fig. 19. A segment of trabecular bone from a median section (IJ) of vertebral body of 89-year-old osteoporotic female subject showing two microcallus patches on horizontal trabeculae

apparent. The most common arrangement in the more anterior part of the middle zone appears to be a honeycomb of tubes of different sizes oriented anterio-posteriorly in the vertebral body. Some of these tubes are open to the anterior wall, and these are essentially tunnels for the entry of blood vessels. The nature of the middle zone varies extensively in different specimens.

A deficiency was usually encountered at the centre of the posterior border of median (IJ) sections (Figs. 7, 11). This is the point of exit of the basivertebral vein or veins. The trabecular plates virtually form the walls of the veins and their tributaries at this region (Figs. 6, 9). Thick plates forming their walls can clearly be identified in other regions of the middle zone (Figs. 6, 7).

The organization observed in coronal (GH) sections through the centres of vertebral bodies is seen in Fig. 3. The clear middle zone with numerous openings for blood vessels and surrounding thick bony plates can again be identified.

Horizontal (CD) sections through the middle zone (Fig. 4) show a honeycomb of bony cylinders of different sizes oriented at right angles to the plane of the section. This implies that the orientation of the plates (really the common walls of adjacent tubes) at the middle zone is mainly vertical. Figure 4 also shows two extensive venous channels with walls consisting of thick bony plates, directed sideways from the centre of the vertebral body.

The variations observed in the middle zones may be due to different patterns of ossification brought about by different patterns of vascular supply during development, whereas the structure of the superior (AB) and inferior (CD) zones seems to be similar in all the specimens (Fig. 5, 17).

Examination of normal bones (Figs. 2, 6) reveals that the number of vertical trabeculae per unit length of the section was much higher in the superior and inferior thirds than in the middle zone. It was rarely possible to trace a vertical trabeculum from one end of the body to the other. The large tubes and plates at the middle zone branch (just as blood vessels branch off from the centre) to give rise to vertical trabeculae at the peripheral zones. In the middle zone of para-sagittal sections (Fig. 10), the vertical arrangement could be more readily identified than in median sections.

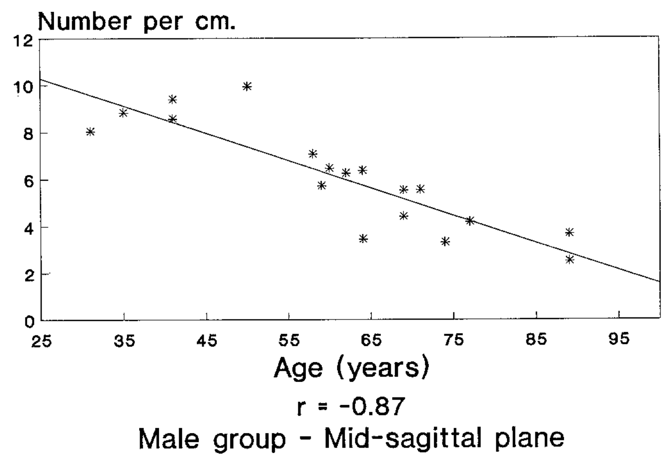
The region of the junction of the vertebral arch with the body is shown in para-sagittal section in Fig. 10 (lower left) and coronal (GH) section in Fig. 9 (top and bottom). Thicker, plate-like structures can be seen in these areas. Vertical trabeculae arise from the respective lateral walls of the vertebral body (Figs. 2, 3, 10), suggesting that loads from the vertebral rim region are transmitted through the lateral walls rather than the central part of the body.

The cancellous structure seems to be more orthogonal towards the vertebral end plates, and double vertebral plates were encountered in many specimens (Figs. 6, 11). These thickened transverse plates below the end plates possibly represent growth arrest lines.

Horizontal elements are, towards the end plates, more or less aligned into horizontal planes. Horizontal (AB, EF) sections (Fig. 5) reveal that these horizontal trabeculae are more plate-like towards the walls of the vertebral body and more rod-like at the centre.

Changes in cancellous structure with increasing porosity

A generalized thinning of trabeculae without disruption of the normal pattern is well seen in many older specimens in median (IJ Fig. 13) and coronal sections (GH Fig. 15). Sections from osteoporotics (both clinically confirmed and histologically identified from loss of bone) showed a change in architecture (Figs. 12, 14, 18). Vertical trabeculae could be traced without much difficulty from one end of the body to the other in the central region (Figs. 12, 14). Here they seem to run approximately parallel to one another, the distance between them being nearly constant. Horizontal trabeculae in the superior and inferior zones markedly increase in length (Figs. 12, 14). The length of the peripheral horizontal trabeculae, shorter in young mature adults, now approaches that of mid-horizontal trabeculae (age series: Figs. 7, 14, 15, 18).



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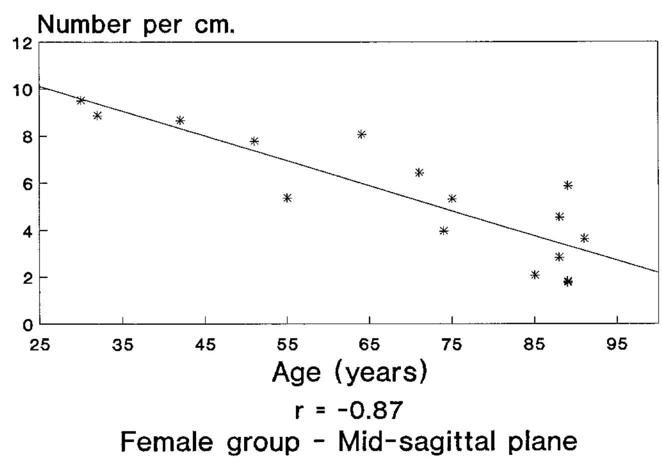

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Fig. 20. Numerical density of horizontal trabeculae in the sagittal plane of fourth lumbar vertebral bodies, males (**a**) and females (**b**)

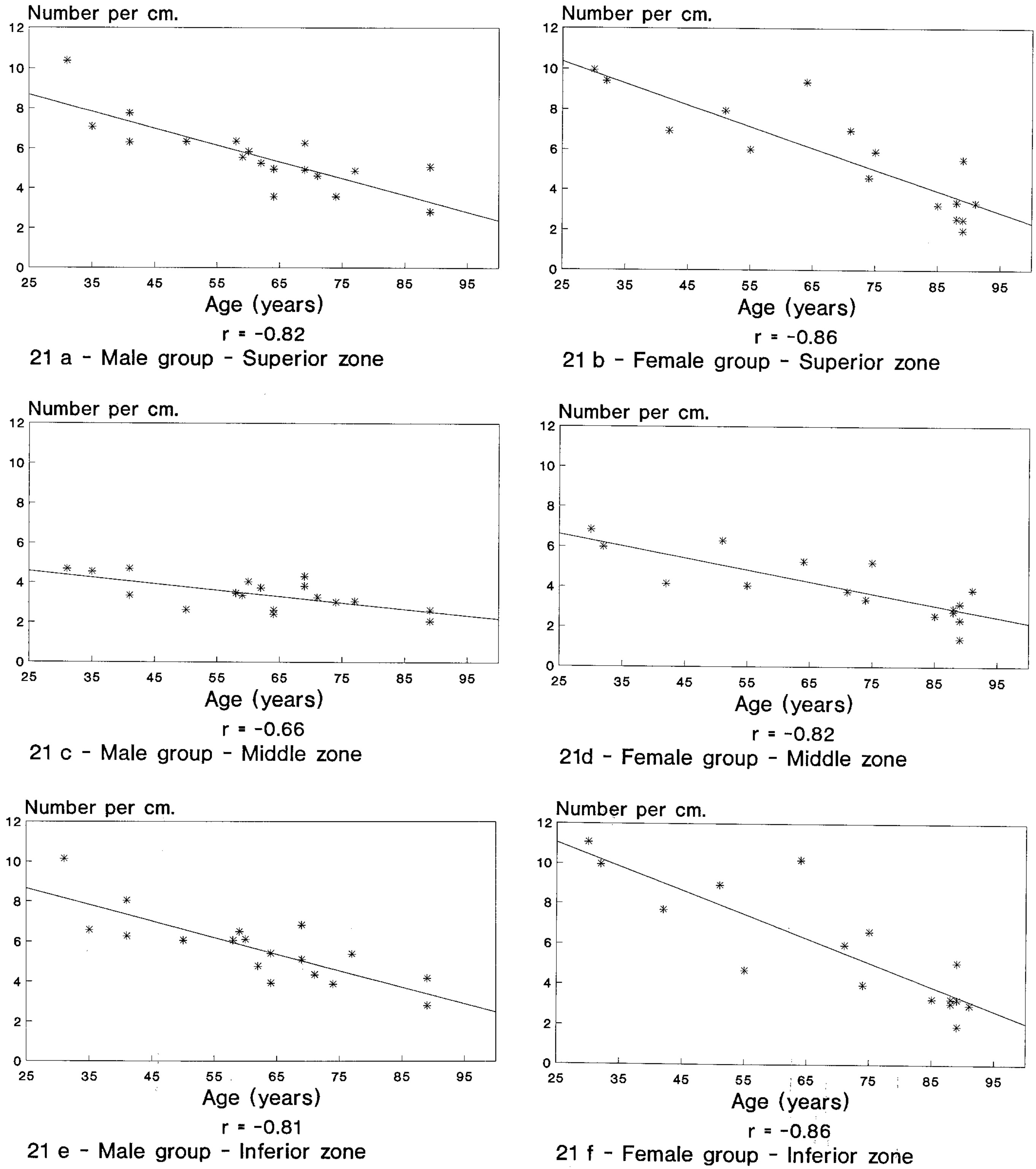

Fig. 21. Numerical density of vertical trabeculae in sagittal plane of fourth lumbar vertebral bodies

Horizontal sections of unembedded bone are difficult to prepare from severely osteoporotic vertebral bodies due to insufficient support for the trabecular elements in this plane. The few horizontal trabeculae that remain in osteoporotic bone are long, thin, frail strands. Most have been completely removed from the periphery, causing increased trabecular separation. Long oblique trabeculae connecting vertical trabeculae can be usually seen at the peripheral regions of osteoporotic bone. In the middle zone, very long horizontal trabeculae are seen in severely osteoporotic sections. Some comparatively thicker plates are still present at the middle zone of some osteoporotic sections (Figs. 12, 18).

In many specimens, isolated depressions in the superior and inferior vertebral end plates were observed from the sixth decade onward (Figs. 10, 15, 18). When typical Schmorl's or other cystic lesions were present, the trabeculae around the lesion were thickened and were structurally different from the rest of the vertebral body.

In many severe osteoporotic cases, large regions devoid of bone were encountered within the vertebral body (Figs. 12, 18). The sections used during this study were prepared very carefully to prevent accidental breaking of the fine trabeculae. Other than the fracture of one corner of the section which occurred during preparation of the sample shown in Fig. 12, very thin trabeculae around the void indicate that this is a genuine, antemortem loss of bone. It should be remarked that the removal of the trabeculae within the vertebral body took place without a collapse in vertebral end plates or walls.

Increases in both trabecular microfractures and free ends were found with increasing age. However, globular bone formation on trabeculae, presumably microcallus, was seen only in individuals above 50 years of age (Fig. 19: it is also present in Figs. 12, 13, 14, 17). Microcallus was seen on vertical, horizontal and even in oblique trabeculae in the older specimens (Fig. 12), but less frequently in the middle third of the vertebral body, where the plates are thick, than in the upper (Fig. 17) and lower thirds where there are more numerous and finer rod-like trabeculae. Microcallus formation was present, however, in all regions of the vertebral body and even in the mid-horizontal trabeculae described earlier.

The morphology of the callus relates to the diameter of the trabeculae on which it formed. On thin (usually horizontal or oblique as in Fig. 19) trabeculae, it presented as small round, fusiform or angulated structures as described by Vernon-Roberts and Pirie (1973). Callus on thick vertical trabeculae (Fig. 14) had a cauliflower appearance, and at higher magnifications showed a large number of perforations. On very thick vertical trabeculae, it presented as aggregates of new tissue on one surface only. In a sample where collapse of the vertebral body had occurred, callus formation was found across the section, linking several trabeculae within the region with so-called bridge callus formation. The formation of fine cancellous networks around existing thick trabeculae was also observed in several old samples.

Table 1 presents ratios of the mean length of horizontal trabeculae in old and osteoporotic samples divided by the mean length in young samples at different zones in both males and females. In the male sample, the range for mid-horizontal trabeculae was 0.67 to 1.21 mm, and at the peripheral zones from 0.47 to 0.78 mm. In the female sample, the length at the middle zone varied from 0.44 to 1.24 mm, and at the periphery from 0.28 to 0.90 mm.

Table 1. Three-dimensional measurements of the length of horizontal trabeculae

Zone	Old/Young		Osteoporotics/Young	
	Male	Female	Male	Female
Superior	1.36	1.48	1.57	2.70
Middle	1.15	1.17	1.29	1.63
Inferior	1.44	2.33	2.56	2.83

In an old, clinically non-osteoporotic male subject, the general pattern of the normal bone was maintained, but the overall length of the trabeculae had markedly increased: the range at the middle zone was from 0.70 to 1.83 mm and at the periphery from 0.54 to 1.4 mm. A clinically non-osteoporotic woman presented a completely different picture: the length of the horizontal trabeculae of the middle zone ranged from 0.73 to 1.33 mm, and at the peripheral zones from 0.63 to 1.78 mm. Thus not only had the normal pattern changed, but the inferior zone consisted of very long horizontal trabeculae when compared with the other two zones.

In the clinically osteoporotic male subject, horizontal trabeculae at the middle zone ranged from 0.65 to 1.78 mm and at the periphery from 0.76 to 2.67 mm, with the inferior zone having the longest trabeculae. In the female osteoporotic, the middle zone trabeculae ranged from 0.98 to 1.83 mm and the peripheral zones from 0.86 to 2.40 mm. The superior and inferior zones in this case had very long horizontal trabeculae compared to the middle zone.

The frequency of vertical and horizontal trabeculae

The relationships between age and the number of horizontal and vertical trabeculae per centimetre are shown in Figs. 20–22. Horizontal trabeculae were counted along the central axis of the vertebral body (plane GH of Fig. 1). There was a highly significant decrease in the number of horizontal trabeculae with age in both males and females ($r = -0.873$, $P < 0.002$ and $r = -0.865$, $P < 0.002$ respectively: Fig. 20a, b).

A highly significant decrease in vertical trabeculae was observed in the superior zone (plane A–B of Fig. 1) in both males and females ($r = -0.816$, $P < 0.002$ and $r = -0.865$, $P < 0.002$ respectively: Fig. 21a, b).

A highly significant decrease was also apparent in the inferior zone (plane EF, Fig. 1) in both sexes ($r = -0.809$, $P < 0.002$ and $r = -0.859$, $P < 0.002$ respectively) (Fig. 21e, f).

In the middle zone (plane C–D of Fig. 1), the decrease in vertical trabeculae with age is greater and more significant in female subjects than males ($r = -0.817$, $P < 0.002$ and $r = -0.662$, $P < 0.01$ respectively: Fig. 21c, d). Within the male group, the decrease in the middle zone is less pronounced than in the other two zones.

Biconcavity index

A significant decrease with age in the biconcavity index was observed in male samples ($r = -0.596$, $P < 0.01$) (Fig. 22a). This decrease was not statistically significant in females ($r = -0.262$: Fig. 22b). This suggests that the decrease in cancellous elements and the change in architecture in a vertebral body is more advanced than would

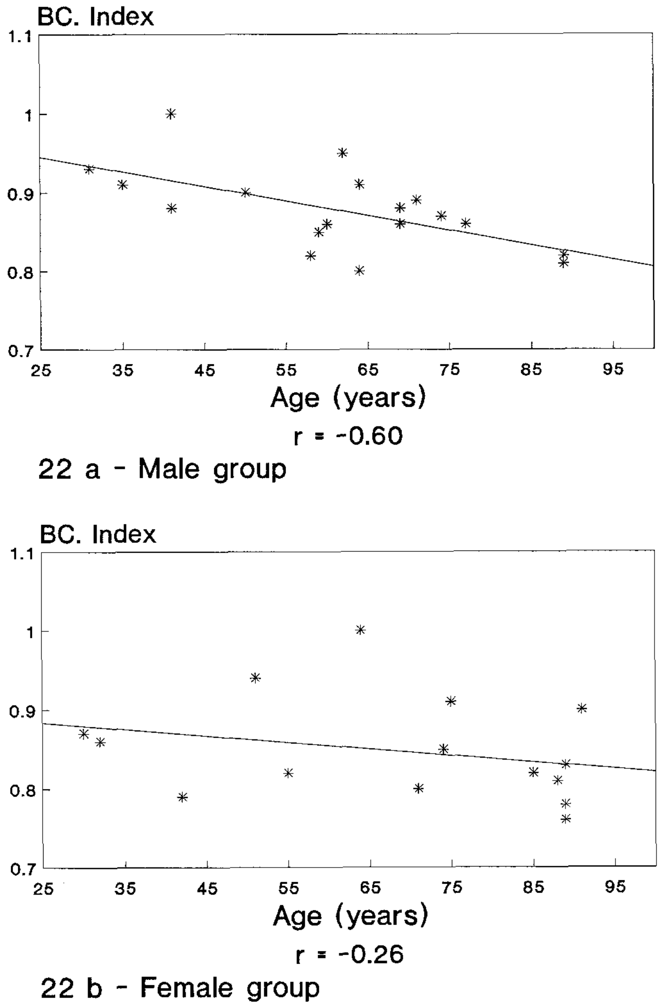

Fig. 22. Biconcavity index of fourth lumbar vertebral bodies in male subjects (**a**) and female subjects (**b**)

be indicated by this index. A vertebral body with a normal biconcavity index could already be advanced in the process of removal of trabecular elements, and a body with a slight decrease in the index may be in an advanced stage of osteoporosis.

Discussion

Our methodology has emphasized the need to study the changing cancellous architecture of ageing and osteoporotic bones with three-dimensional methods. Thick sections provided a volume of spongy bone adequate to show the distribution and organization of the bone at the intermediate level of organization: they kept in their anatomical context regions of surface activity that could be studied at high magnification by SEM. This method is complementary to other techniques, such as continuous rotation parallax of 4-mm-diameter beams cut in orthogonal planes from different anatomical regions of these bones (Boyde et al. 1989, 1990), two-dimensional fast Fourier transform analysis of x-ray and optical images (Jayasinghe and Boyde 1990) and SEM (Jayasinghe et al. 1993).

The measurement of the true length of the trabecular rods gave values that refine our analysis of the spatial frequency domain of trabecular spacing. Irrespective of vertebral body size, very long horizontal trabeculae could usually be observed in old and osteoporotic samples; no correlation was detected between vertebral body size and the length of the horizontal trabeculae in normal samples of younger age.

The number and size of the vertical trabeculae are determined by the original pattern of ossification and growth, in turn dependent on the pattern of the vasculature of the vertebral body which may not relate to vertebral body size.

Vertebral bodies have remarkably thin cortices (Eastell et al. 1990), well shown in the 3D views of the thickly sliced material. In younger samples, we found that the vertebral rim mostly consisted of thick continuous bone; in many old specimens, thinning and thinning out (trabeculization) of the rim had taken place so that it was hardly recognizable. This partly explains the reduced radiodensity of the rim seen in older individuals (Schmorl and Junghans 1975).

Our observations support those of Arnold (1973) who found that biconcavity in the vertebral body appears to increase if there is a decrease in cancellous bone, but is not related to whether or not collapse fractures are present. He proposed that biconcavity develops progressively once bone loss has occurred and is, therefore, a measure of the duration of an osteoporotic state rather than an index of its presence.

Clinically, osteoporosis means that the subject has a lower bone mass than might be expected from age and sex norms, and an increased risk of fractures (Woolf and Dixon 1988). The disease state is usually identified by spontaneous fractures of the vertebrae or long bones.

Arnold (1973) concluded that less than 0.07 g ash/cm^3 of medullary tissue represents a pathological level that can be termed the osteoporotic state. However, not all patients below the osteoporotic threshold had fractures, and half of those with a single fracture in the lower spine had a normal vertebral mineral content. At present, bone-mass measurements are used to diagnose the osteoporotic state, but there is an extensive overlap in bone mass values between normal and osteoporotic persons (Heaney 1989), and the expected correlation between the severity of osteoporosis and the degree of bone loss may be absent (Cummings 1987; Pødenphant et al. 1987). Decreased bone mineral density may also play an important role, together with the increased risk of falling and patterns of falling with age (Cummings 1987).

Heaney (1987, 1989) suggested that fatigue damage, bone quality and trabecular connectivity are important factors that contribute to increased bone fragility and osteoporotic fractures. As the age-related decline in mechanical competence of cancellous bone exceeds that expected from the age-related decline in mass, the alteration in architecture, undetected by simple bone-mass measurements, must be important (Kleerekoper et al. 1987).

Although the changing cancellous bone architecture during ageing and osteoporosis is clearly important, the exact ways in which these changes are brought about has proved difficult to describe and quantitate for different conditions (Arnold 1970), ages and sexes (Aaron et al. 1987; Compston et al. 1987). Mainly on the basis of 2D analyses, it has been assumed that the structural integrity of trabecular bone is preserved in men, but not in women. Contrary to the above views, uniform thinning without removal of trabecular elements was observed in the present 3D study in both old men and women. The conflicting findings may result from the surface picture being misleading as to the nature of the 3D structure (Hahn et al. 1989, 1992).

We found that extensive regional variations exist in mid-sagittal (IJ) sections of a vertebral body; plate or tube-like regions (usually termed normal bone) and regions of rod or needle-like elements (as in osteoporotic bone) occurred in the same vertebral section. In a comparative study, therefore, the same region should be compared in different specimens. Due to extensive developmental variations, the normal regional structure will vary most widely in the middle zone, but our findings suggest that developmental variation may make histomorphometric analysis, even of a whole vertebral section, unsatisfactory.

Development, the mechanical loading history, and functional importance may be some of the factors that decide variations in the pattern of trabecular bone architecture between sites. With ageing, the effect of systemically acting factors may be uniform on all these sites, but locally acting factors could be very different. While measurement of bone mass (trabecular bone volume) at a site such as the iliac crest might give some indication about bone loss in the spine, architectural changes such as trabecular thinning and loss of connectivity in the iliac crest might not give correct information about analogous changes in the spine. Our observations confirm the view that loss of connectivity can only be properly assessed with 3D evaluation.

Bone loss is believed to occur by conversion of plates to rods, followed by complete removal of the trabecular elements, with the distance between the vertical trabeculae showing a highly significant age-related increase (Mosekilde 1988). However, Vogel et al. (1989) have shown that the fractional bone volume depends almost entirely on the number of plates, whereas there is no relation, except perhaps an inverse one, between bone volume and the number of rods. Hahn et al. (1989) also found that the number of rod-like trabeculae is constant in every decade, and considered the appearance of perforations to be the main mechanism for conversion of plates to rods.

Reduction in the number of plates with increasing age could easily be observed in our thick sections. However, no method has yet been developed to quantify this reduction, because of the extensive variations in the form of trabecular plates in different samples. Plates of different sizes usually exist in normal bone, and it is impossible to draw a line which will distinguish plates from rods. [Our continuous rotation data (Boyde et al. 1989, 1990) show that a large proportion of 'rods' could be regarded as narrow plates]. Furthermore, different numbers of horizontal plates usually exist, and they appear as rods in sagittal sections, commonly being encountered under the vertebral end plates. Due to nonuniformity in loss of rods, it is also impossible to quantify their disappearance. Similarly, it is difficult to quantify the appearance and progression of perforations on the plates and rods. All this emphasizes the need for an analysis of 3D architecture and connectedness; it may be pointless to quantify bits and pieces of cancellous bone unless they are seen in context.

The total removal of trabeculae during osteoporosis may be preceded by generalized thinning of trabeculae (and of cortical bone) in both sexes, as in age-related (Type II) osteoporosis (Riggs and Melton 1983). In gonadal (Type I) osteoporosis, the complete removal of trabecular elements may occur in association with a higher rate of bone resorption at a much younger age than in normal females, with uniform thinning prior to complete removal unseen. Failure to make this distinction may have led to the belief that the main mode of bone loss in females is always by complete removal of trabecular elements.

What are the possible mechanisms responsible for selective removal of trabeculae? Arnold (1970) pointed out that the action of a local factor may be more important for focal excessive resorption than systemically acting factors and mechanical loading. The effect of systemically acting endocrine and nutritional factors may be diffuse (if close proximity of a piece of bone to a blood vessel is not considered) on all the surfaces of cancellous bone. The role of decreased mechanical stress in explaining the excessive resorption appears more likely to be secondary rather than primary. Local factors that change the micro-environment of the trabeculae include changes in vascularity with age; changes in pressure related, for example, to tumours; or the production of different agents (such as cytokines) by the local cell community.

Some controversy exists as to whether the thickness of the remaining vertical trabeculae increases with age in response to the increased mechanical loading which they have to bear (Atkinson 1967; Pesch et al. 1977; Twomey et al. 1983; Bergot et al. 1988; Mosekilde 1988). We found evidence for compensatory thickening of vertical trabeculae in many specimens during the present study, even when normal variation was taken into account. According to Frost (1964), trabecular bone is used to withstand pure compression. He stated that cross-sectional growth in size is controlled by the sizes of the bending loads, unequal and increasing loads causing increase in cross-sectional size (Frost 1963, 1964). Frost considered that increasing surface concavity as load is applied causes local gain, while increasing convexity causes loss of bone. If this explanation is correct, the remaining vertical trabeculae at the periphery would increase in thickness to bear the additional load.

Arnold (1968) proposed that a peripheral extension of the large-diameter cylinders from the middle zones occurred to replace the lost transverse plates of the end zones. However, our findings were consistent with a process of thinning of the bony cylinders at the centre and thickening of vertical trabeculae at the periphery to produce more or less uniformly thickened vertical trabeculae extending from one end plate to the other.

Collapse has usually been reported to occur first at the central region of the vertebral body underneath the area adjacent to the nucleus pulposus (Twomey et al. 1983). In our study, the area below the vertebral rim of the osteoporotic samples showed vertical trabeculae connected to the lateral walls as in normal bone. Even in severe osteoporotic cases, loads could still be transferred to the lateral walls. With the architectural changes observed, the central region becomes the weakest part of the body, perhaps explaining why collapse occurs first there.

In the present study, trabecular thinning was first observed around the region of the entry of the basivertebral vessels to the vertebral body. It has been reported that the basivertebral vein becomes enlarged in advanced age (Williams et al. 1989). This raises the question whether this thinning occurs in response to enlarging veins or is a truly independent age change.

Much evidence suggests that the vascular changes that take place during ageing and the development of osteoporosis may contribute to the observed structural changes in cancellous bone (Batson 1956; Macnab 1977; Arnoldi et al. 1972; Farfan 1975; Arnoldi 1976; Crock and Yoshizawa 1977; Helfet and Grubel Lee 1978; Ratcliffe 1982, 1986; Matsuo 1985). Intra-osseous hypertension may be a major factor contributing to the resorption of trabeculae at the focal level. The collapse of the already weakened trabecular network within the vertebral body might take place during a sudden rise in intra-osseous venous pressure without a collapse of the walls of the vertebral body. Future research into structural changes of cancellous bone should give proper attention to the effect of changing vascularity on the vertebral body, including intra-osseous hypertension and venous stasis.

Our observations concerning trabecular microcallus formation confirm previous reports (Vernon-Roberts and Pirie 1973; Hansson 1977). Hahn et al. (1989) have suggested that the appearance of microcallus may be an indication of changed stability of the vertebral body through loss of structure and quality of bone. The loss of connectivity would result in greater fragility (Heaney 1989) and accumulation of microdamage (Frost 1973, 1985). Whilst microcallus formation may show compensatory possibilities of bone that is still intact (Hahn et al. 1989), it is doubtful whether bridge callus formation, observed in this study only where collapse or wedging of the vertebra had taken place, would form a functionally effective replacement structure.

In conclusion, we have shown that a simple optical method can provide a contextural image of bone that is valuable for illustrating and understanding the changes that occur in tissue tectonics with age and pathology.

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References

Aaron JE, Makins NB, Sagreiya K (1987) The microanatomy of trabecular bone loss in normal aging men and women. Clin Orthop Rel Res 215:260–271

Amstutz HC, Sissons HA (1969) The structure of the vertebral spongiosa. J Bone Joint Surg 51 B:540–550

Arnold JS (1968) External and trabecular morphologic changes in lumbar vertebrae in aging. In: Whedon GD, Cameron JR (eds) Progress in methods of bone mineral measurement. US Dept. of Health, Education and welfare, Bethesda, MD, pp 352–411

Arnold JS (1970) Focal excessive endosteal resorption in aging and senile osteoporosis. In: Barzel US (ed) Osteoporosis. Grune & Stratton, New York, pp 80–100

Arnold JS (1973) Amount and quality of trabecular bone in osteoporotic vertebral fractures. Clin Endocrinol Metab 2:221–238

Arnold JS, Bartley MH, Tont SA, Jenkin DP (1966) Skeletal changes in aging and disease. Clin Orthop Rel Res 49:17–38

Arnoldi CC (1976) Intraosseous hypertension. Clin Orthop Rel Res 115:30–34

Arnoldi CC, Linderholm H, Müssbichler H (1972) Venous engorgement and intraosseous hypertension in osteoarthritis of the hip. J Bone Joint Surg 54B:409–421

Atkinson PJ (1967) Variation in trabecular structure of vertebrae with age. Calcif Tissue Res 1:24–36

Batson OV (1956) The vertebral vein system. Am J Roentgenol 78:195–212

Bell GH, Dunbar O, Beck JS (1967) Variation in strength of vertebrae with age and their relation to osteoporosis. Calcif Tissue Res 1:75–80

Bergot C, Preteux F, Laval-Jeantet AM (1987) Quantitative image analysis of thin sagittal and transversal slices from autopsy specimens of L3 vertebrae. In: Christiansen C, Johansen JS, Riis BJ (eds) Osteoporosis 1987. Osteopress ApS, Copenhagen, pp 338–340

Bergot C, Laval-Jeantet AM, Preteux F, Meunier A (1988) Measurement of quantitative image analysis. Calcif Tissue Res 43:143–149

Birkenhäger-Frenkel DH, Courpron P, Hupscher EA, Clermonts E, Coutinho MF, Schmitz PIM, Meunier PJ (1988) Age-related changes in cancellous bone structure. Bone Miner 4:197–216

Boyde A, Jayasinghe JAP, Jequier B (1989) Three-dimensional integram images of trabecular bone. Bone 10:12

Boyde A, Radcliffe R, Watson TF, Jayasinghe JAP (1990) Continuous motion parallax in the display and analysis of trabecular bone structure. Bone 11:228

Cann CE, Genant HK, Kolb FO, Ettinger B (1985) Quantitative computed tomography for prediction of vertebral fracture risk. Bone 6:1–7

Compston JE, Mellish RWE, Garrahan NJ (1987) Age-related changes in iliac crest trabecular microanatomic bone structure in man. Bone 8:289–292

Cosman F, Schnitzer MB, McCann PD, Parisien MV, Lindsay R (1992) Relationship between quantitative histological measurements and non-invasive measurements of bone mass. Bone 13:237–242

Crock HV, Yoshizawa H (1977) The blood supply of the vertebral column and spinal cord in man. Springer, Heidelberg Berlin New York

Cummings SR (1987) Epidemiology of hip fractures. In: Christiansen C, Johansen JS, Riis BJ (eds) Osteoporosis 1987. Osteopress ApS, Copenhagen, pp 40–44

Delling G (1989) Neuere Vorstellungen zur Bau und Struktur der menschlichen Spongiosa – Ergebnisse einer kombinierten zwei- und dreidimensionalen Analyse. Z Gesamte Inn Med 44:536–540

Dunnill MS, Anderson JA, Whitehead R (1967) Quantitative histological studies on age changes in bone. J Pathol 94:275–291

Eastell R, Mosekilde L, Hodgson SF, Riggs BL (1990) Proportion of human vertebral body bone that is cancellous. J Bone Miner Res 5:1237–1241

Farfan HF (1975) Muscular mechanisms of the lumbar spine and the position of power and efficiency. Orthop Clin North Am 6:135

Feldkamp LA, Goldstein SA, Parfitt AM, Jesion G, Kleerekoper M (1989) The direct examination of three-dimensional bone architecture in vitro by computed tomography. Bone 4:3–11

Frost HM (1963) Bone remodelling dynamics. Thomas, Springfield, Ill

Frost HM (1964) The laws of bone structure. Thomas, Springfield, Ill

Frost HM (1973) The spinal osteoporosis. Clin Endocrinol Metab 2:257–275

Frost HM (1985) Pathomechanics of osteoporosis. Clin Orthop 200:198–225

Galante J, Rostoker W, Ray RD (1970) Physical properties of trabecular bone. Calcif Tissue Res 5:236–246

Garrahan NJ, Mellish RWE, Compston JE (1986) A new method for the two-dimensional analysis of bone structure in human iliac crest biopsies. J Microsc 142:341–349

Hahn M, Vogel M, Pompesius-Kempa M, Delling G (1989) Kombinierte zwei- und dreidimensionale Analyse der Wirbelsäule als Grundlage für das Verständnis endokriner Knochenmassenverlust-Syndrome. Quintessenz, Berlin

Hahn M, Vogel M, Pompesius-Kempa M, Delling G (1992) Trabecular bone pattern factor – a new parameter for simple quantification of bone microarchitecture. Bone 13:327–330

Hansson T (1977) The bone mineral content and biomechanical properties of lumbar vertebrae. Monograph, Dept. Orthopaedic Surgery, University of Göteborg, Sweden

Heaney RP (1987) Qualitative factors in osteoporotic fracture: the state of the question. In: Christiansen C, Johansen JS, Riis BJ (eds) Osteoporosis 1987. Osteopress ApS, Copenhagen, pp 281–287

Heaney RP (1989) Osteoporotic fracture space: an hypothesis. Bone Miner 6:1–13

Helfet AJ, Grubel Lee DM (1978) Disorders of the lumbar spine. Lippincott, Philadelphia Toronto

Jayasinghe JAP, Boyde A (1990) A preliminary study of normal and osteoporotic trabecular bone using frequency domain analysis. Bone 11:227

Jayasinghe JAP, Jones SJ, Boyde A (1993) Scanning electron microscopy of human vertebral trabecular bone surfaces. Virchows Arch [A] 422:25–34

Jensen KS, Mosekilde L, Mosekilde L (1990) A model of vertebral trabecular bone architecture and its mechanical properties. Bone 11:411–415

Kleerekoper M, Villanueva AR, Staneiu J, Sudhaker Rao D, Parfitt AM (1985) The role of three-dimensional trabecular microstructure in the pathogenesis of vertebral compression fractures. Calcif Tissue Int 37:594–597

Kleerekoper M, Feldkamp LA, Goldstein SA, Flynn MJ, Parfitt AM (1987) Cancellous bone architecture and bone strength. In: Christiansen C, Johansen JS, Riis BJ (eds) Osteoporosis 1987. Osteopress ApS, Copenhagen, pp 294–300

Lindahl O, Lindgrén GH (1962) Grading of osteoporosis in autopsy specimens. Acta Orthop Scand 32:85–100

Macnab I (1977) Backache. Wiliam & Wilkins, Baltimore

Martin DB (1966) Stereoscope with optical means of measuring parallax. Int Arch Photogrammetry 16:37–44

Matsuo H (1985) Venographic findings on changes in the lumbar anterior internal vertebral vein with regard to age. Nippon Ika Daigaku Zasshi 52:633–641

Mosekilde L (1988) Age-related changes in vertebral trabecular bone architecture – assessed by a new method. Bone 9:247–250

Mosekilde L (1990) Consequences of the remodelling process for vertebral trabecular bone structure: a scanning electron microscopy study (uncoupling of loaded structures). Bone Miner 10:13–35

Mosekilde L, Mosekilde L, Danielsen CC (1987) Biomechanical competence of vertebral trabecular bone in relation to ash density and age in normal individuals. Bone 8:79–85

Odgaard A, Gundersen HJG (1993) Quantification of connectivity in cancellous bone, with special emphasis on 3-D reconstructions. Bone 14:173–182

Parfitt AM, Mathews CHE, Villanueva AR, Kleerekoper M, Frame B, Rao DS (1983) Relationship between surface, volume, and thickness of iliac trabecular bone on aging and in osteoporosis: implications for the microanatomic and cellular mechanism of bone loss. J Clin Invest 72:1396–1409

Pesch HJ, Henschke F, Seibold H (1977) Einfluss von Mechanik und Alter auf den Spongiosaumbau in Lendenwirbelkörpern und im Schenkelhals. Virchows Arch A Pathol Anat Histopathol 377:27–42

Pødenphant J, Herss Nielsen VA, Riis BJ, Gotfredsen A, Christiansen C (1987) Bone mass, bone structure and vertebral fractures in osteoporotic patients. Bone 8:127–130

Pompesius-Kempa M, Hahn M, Vogel M, Delling G (1989) Neue Untersuchungen zur Mikroarchitektur der Spongiosa bei Osteopathien im Vergleich zu altersbedingten Veränderungen. In: Willert HG, Heuck FHW (eds) Neuere Ergebnisse in der Osteologie. Springer, Heidelberg Berlin New York

Pugh JW, Rose RM, Radin EL (1973) Elastic and viscoelastic properties of trabecular bone: dependence on structure. J Biomech 6:475–485

Ratcliffe JF (1982) An evaluation of the intra-osseous arterial anastomoses in the human vertebral body at different ages. A microarteriographic study. J Anat 134:373–382

Ratcliffe JF (1986) Arterial changes in the human vertebral body associated with aging. Spine 11:235–240

Riggs BL, Melton LJ (1983) Evidence for two distinct syndromes of involutional osteoporosis. Am J Med 75:899–901

Schmorl G, Junghans H (1975) The human spine in health and disease. Grune & Stratton, New York

Singh I (1978) The architecture of the cancellous bone. J Anat 127:305–310

Twomey L, Taylor J, Furniss B (1983) Age changes in the bone density and structure of the lumbar vertebral column. J Anat 136:15–25

Vernon-Roberts B, Pirie CJ (1973) Healing trabecular microfractures in the bodies of lumbar vertebrae. Ann Rheum Dis 32:406–412

Vesterby A (1990) Star volume of marrow space and trabeculae in iliac crest: sampling procedure and correlation to star volume of first lumbar vertebra. Bone 11:149–155

Vesterby A, Gundersen HJG, Melsen F (1989) Star volume of marrow space and trabeculae of the first lumbar vertebra: sampling efficiency and biological variation. Bone 10:7–13

Vogel M, Hahn M, Pompesius-Kempa M, Delling G (1989) Trabecular microarchitecture of the human spine. In: Willert HG, Heuck FHW (eds) Neuere Ergebnisse in der Osteologie. Springer, Heidelberg Berlin New York, pp 449–455

Wakamatsu E, Sissons HA (1969) The cancellous bone of the iliac crest. Calcif Tissue Res 4:147–161

Whitehouse WJ, Dyson ED, Jackson CK (1971) The scanning electron microscope in studies of trabecular bone from a human vertebral body. J Anat 108:481–496

Williams PL, Warwick R, Dyson M (eds) (1989) Gray's Anatomy, 37th edn. Churchill Livingstone, Edinburgh

Woolf AD, Dixon ASt.J (1988) Osteoporosis – a clinical guide. Dunitz, London