FROM THE ORTHOPAEDIC CLINIC, (HEAD: PROFESSOR STEN FRIBERG), KAROLINSKA INSTITUTET, AND THE DEPARTMENT OF PATHOLOGY (HEAD: ÅKE G. H. LINDGREN), ST. GÖRAN'S HOSPITAL

GRADING OF OSTEOPOROSIS IN AUTOPSY SPECIMENS

A New Method

By

OLOV LINDAHL and ÅKE G. H. LINDGREN

Whereas in advanced osteoporosis there is little difficulty in forming a diagnosis by radiography, the mild case is not so easily recognized; by the time the condition can be detected much of the bone has disappeared; the actual amount is put at between 15 and 60 per cent by various workers (3, 6, 8, 12). The lower values apply to small bones with little soft-tissue covering, and the higher values to the spinal column and the pelvis. Nor is radiography a reliable method of grading the porosity or of evaluating the changes therein. Moreover, at least in the milder grades of osteoporosis, macroscopic and histologic evaluation of the bone specimen is extremely difficult and purely subjective; one reason for this is that the porosity of spongy bone varies widely throughout the skeleton.

In anthropology it was found that the quotient of weight by volume of various parts of the skeleton decreased significantly with the age of the subject. It was higher for negroes than for whites of the same age. The following average values were obtained for 80 femurs and vertebral bodies (5).

	Negroes		Whites	
	Men	Women	Men	Women
Femur	0.70	0.65	0.63	0.60
Vertebrae	0.48	0.46	0.41	0.38

Density, or true density, (weight/volume) is a familiar physical constant and the term applies to materials of uniform density. *Apparent density* is a similar constant used of porous materials such as plastic

sponge. It, too, is the quotient of weight by volume, but, unlike the true density, the small spaces in the material are included in the volume.

For compact bone, the true and apparent densities are identical and the values are the same for osteoporotic as for normal bone. In the case of spongy bone, however, the true density is the same for the normal and osteoporotic tissue, and equal to that for compact bone. The apparent density is considerably lower for spongy bone, and it decreases further as the porosity increases. Determination of the apparent density of spongy bone would thus seem to be a highly reliable test for osteoporosis and at the same time to provide a suitable means of grading the disease. In osteoporosis there is probably a change in the thickness and amount of compact bone, but this cannot be determined by the methods discussed here, because there is no difference in the apparent density for the normal and pathologically altered tissue. No systematic investigation seems to have been made of the apparent density of spongy bone, with a comparison between normal subjects and osteoporotics; nor are there data on the normal ranges of variation. The present study was accordingly undertaken with a view to determining the apparent density of spongy bone at various ages and examining the different grades of osteoporosis.

MATERIAL

The specimens of bone were taken from the lumbar spine and the upper part of the tibia of 60 autopsy cases of various ages. As far as possible each decade was represented by 8 subjects, 4 of each sex, and a selection was made of persons who had died without prolonged confinement to bed. At St. Göran's Hospital, where there are about 1000 beds and where some 600 autopsies are performed every year, the autopsy cases are predominantly of the higher age groups. Since ages below 40 years were represented to but a small extent during the two years covered by the investigation (1958–59), the material was supplemented with specimens obtained at medico-legal autopsies, where the cause of death was usually external violence or, occasionally, poisoning.

The age and sex distributions of the material are given in Table 1. Women were slightly in the minority in the lower age groups. Most of the subjects below 40 years of age were medico-legal cases (Table 2). The younger age groups therefore consisted chiefly of physically healthy persons, whereas the older subjects had suffered from, and died from, disease. For this reason, and because the number of subjects in each

group was too small, the material so obtained cannot be regarded as statistically representative of the general population in respect of the condition of the bone. A more representative series was not, however, available.

TABLE 1

Distribution of subjects with respect to sex and age.

Age group	14-19	20-29	30-39	40-49	50-59	60-69	70-79	80-91	Total
Women	4	1	2	4	3	3	5	7	29
Men	3	6	5	3	3	3	4	4	31
Total	7	7	7	7	6	6	9	11	60

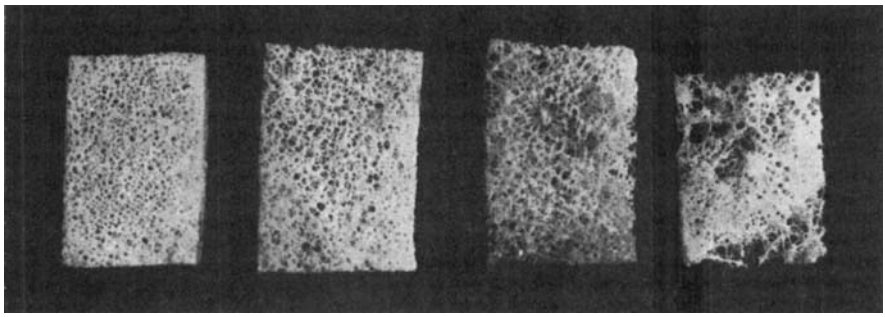
TABLE 2

Distribution of subjects with respect to age group and type of autopsy (clinical or medico-legal).

Age group	14-19	20-29	30-39	40-49	50-59	60-69	70-79	80-91	Total
Medico-legal	6	7	3	0	0	0	0	0	16
Clinical	1	0	4	7	6	6	9	11	44

METHODS

Macroscopic evaluation.—The macroscopic grading of porosity was performed on a cut surface of each bone specimen. The following four-degree scale was applied according to the degree of osteoporosis: normal, mild, moderate, severe (Fig. 1).



Normal Mild Moderate Severe

Fig. 1.

Specimens of spongy bone from lumbar vertebrae with different grades of porosity, classed according to four-degree scale of osteoporosis.

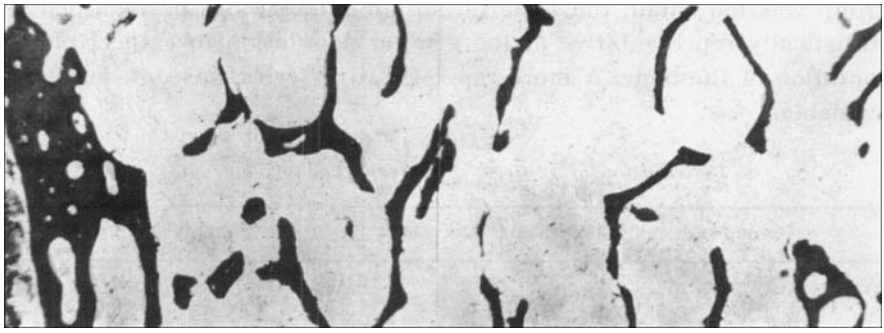


Fig. 2.
Microradiograph of specimen of tibia containing an area of cortical bone and spongy bone, used for determining the relative area of bone in the section.

Microscopic evaluation.—From a tibia of each subject one bone specimen was resected 5 cm from the knee-joint and perpendicular to the cortical bone. The specimens were fixed in formalin, decalcified, stained by the van Gieson and haematoxylin-eosin methods and sectioned.

Determination of the relative area of bone in the section.—A photomicrograph was taken of the microscope preparation and enlarged to 24×36 cm. to give a magnification of 60 of the original bone. The photomicrograph included the cortical bone and the adjacent spongy bone. The relative area of bone in the section was determined by cutting out the parts of the print containing the cortical bone and trabeculae of the spongy bone and weighing them. The value so obtained was expressed as a percentage of the weight of the whole print (Fig. 2).

Determination of the apparent density.—From the removed bones, rectangular specimens were cut with a band saw in mutually perpendicular planes so that they consisted only of spongy bone, with no soft parts or cortical bone. One such specimen was obtained from the tibia and one from each of 1–4 lumbar vertebrae. The approximate size of the tibia specimens was 20 × 25 × 40 mm and that of the vertebrae specimens 15 × 20 × 30 mm.

By repeated vigorous rinsing in hot water these specimens were freed from blood, marrow and most of the fat. They were then yellowish-white or white, and in only a few cases was there any trace of blood. The specimens were measured with sliding calipers in three mutually perpendicular directions and were left for 48 hours in clean water to remove any residual blood. After drying they were extracted with

xylene, dried again at 100° C for 3 days, and weighed. The apparent density was then determined by dividing the weight by the volume. It was thus expressed in milligrammes per cubic millimetre.

Statistics.—The usual statistical methods were used for calculating the mean, range, standard error and regression coefficient. The formulae and methods are reported elsewhere (10).

RESULTS

Macroscopic examination.

Osteoporosis visible to the naked eye did not appear until after 50 years of age; it was distinctly more marked among the women (Table 3, Fig. 3).

Microscopic examination.

No attempt was made to grade the osteoporosis in the sections under the microscope (see *Relative area of bone*). The sections were examined only for the presence of pathologic alterations. In no case were osteomalacia or tumours found.

Relative area of bone.

The mean relative area of bone in different age groups decreased with age, but this reduction was not regular (Table 4, Fig. 4). There was no difference between the sexes, as there was in the case of the gross examination.

There was a significant correlation between age and relative area of bone (regression coefficient -0.131 ± 0.047; $0.001 < P < 0.01$). The graphic representation of the correlation between apparent density and

TABLE 3

Distribution of subjects according to degree of osteoporosis, evaluated by gross examination.

Age group	14-19		20-29		30-39		40-49		50-59		60-69		70-79		80-91		Total		
Sex	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀+♂
Degree of osteoporosis																			
None	4	3	2	5	2	5	4	3	0	3	0	0	0	0	0	0	11	19	30
Mild	0	0	0	0	0	0	0	0	1	0	0	0	0	2	0	0	1	2	3
Moderate	0	0	0	0	0	0	0	0	2	0	0	3	3	2	0	2	5	7	12
Severe	0	0	0	0	0	0	0	0	0	0	3	0	2	0	7	2	12	2	14

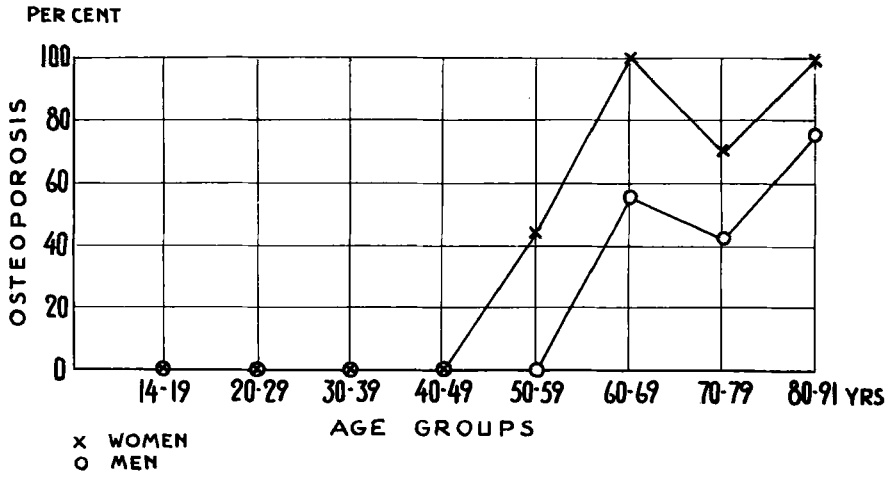


Fig. 3.

Variation of number of cases of osteoporosis with respect to age group. The degree of osteoporosis was evaluated by gross examination. The percentage of subjects with moderate osteoporosis has been divided by 2 and that for mild osteoporosis by 3; the percentages in each age group have then been added. Thus, 100 per cent osteoporosis corresponds to the situation where all the subjects in the age group have severe osteoporosis.

relative area of bone shows a wide scattering of the values (Fig. 5). The correlation was almost significant (regression coefficient $+0.245 \pm 0.119$; $0.01 < P < 0.05$).

Apparent density.

Variation between lumbar vertebrae of the subject.—One to four vertebrae were examined in each of 60 subjects: in 7 subjects one vertebra, in 8 two, in 38 three and in 7 subjects four, a total of 165 vertebrae. In only 9 per cent of the subjects did the apparent density of the

TABLE 4

Mean relative area of bone from tibia in the various age groups (expressed as a percentage of the total area of section).

Age group	14-19	20-29	30-39	40-49	50-59	60-69	70-79	80-91
Mean area of bone								
Female	26	23	26	22	16	14	13	20
Male	29	21	26	22	12	21	20	17
Both sexes	27	21	26	22	14	18	16	18

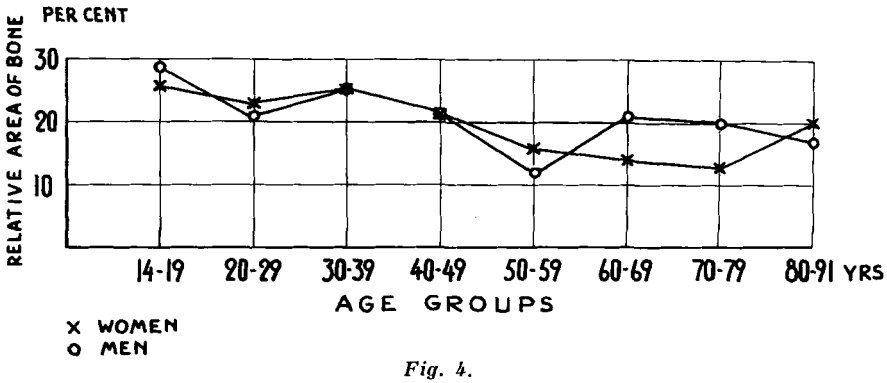


Fig. 4.

Variation of mean relative area of bone in the tibia section with respect to age group.

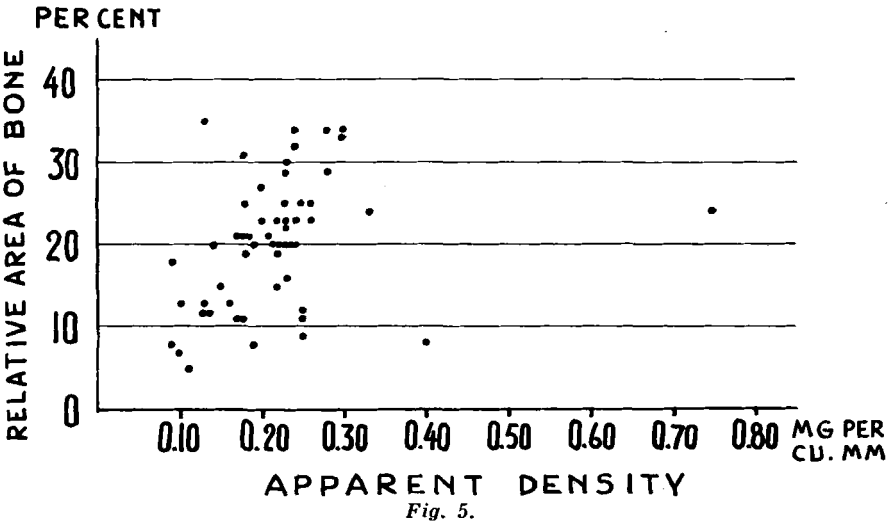


Fig. 5.

Relationship between apparent density and relative area of spongy bone from the tibia. Each dot represents one subject.

TABLE 5

Distribution of subjects according to difference in apparent density between vertebrae of the same subject.

Difference (mg/cu.mm)	0.00	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09
No. of subjects										
2 vertebrae	2	3	1	1	1	0	0	0	0	0
3 vertebrae	4	11	7	6	6	0	1	0	1	2
4 vertebrae	0	4	2	0	0	0	1	0	0	0

vertebrae differ by more than 0.04 mg per cu. mm (Table 5). This suggests that the apparent density of the lumbar vertebrae of a particular subject is largely the same, within the limits of the error of the method. In what follows, therefore, the mean apparent density of all the vertebrae for the individual subject will be used.

Variation between lumbar vertebrae and tibia.—In most cases the differences between the specimens of tibia and vertebrae from a particular subject were small, but they were considerably greater than the differences between the lumbar vertebrae, and exceeded 0.04 mg per cu. mm in 35 per cent of the subjects (Table 6). There was a highly significant correlation between the apparent density of the vertebrae and that of the tibia (regression coefficient $+0.914 \pm 0.140$; $P < 0.001$). The gradient

TABLE 6

Distribution of subjects according to difference in apparent density between vertebrae and tibia.

Difference (mg/cu.mm)	0.00	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09	0.10	>0.10
No. of subjects	2	12	9	9	4	5	4	2	2	2	1	3

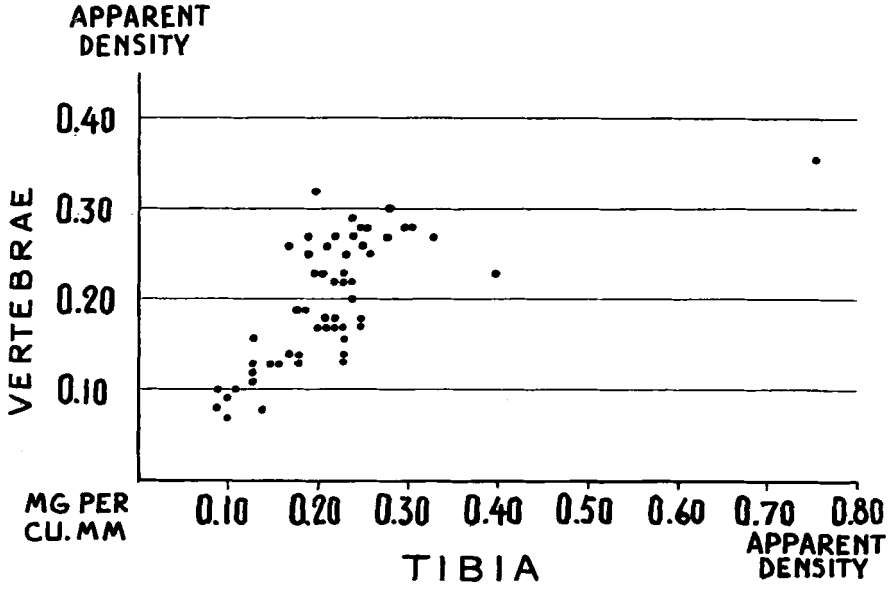


Fig. 6.

Relationship between apparent density of vertebrae and tibia of the same subject. Each dot represents one subject.

of the regression line for the values higher than 0.20 is steeper than for lower values. (These two groups above and below 0.20 mg per cu. mm are later defined as normal and osteoporotic, respectively). This may be interpreted as showing that the apparent density of the tibia did not decrease as rapidly as that of the vertebrae in cases of osteoporosis. This is also suggested by the graphical representation of the relationship in Fig. 7.

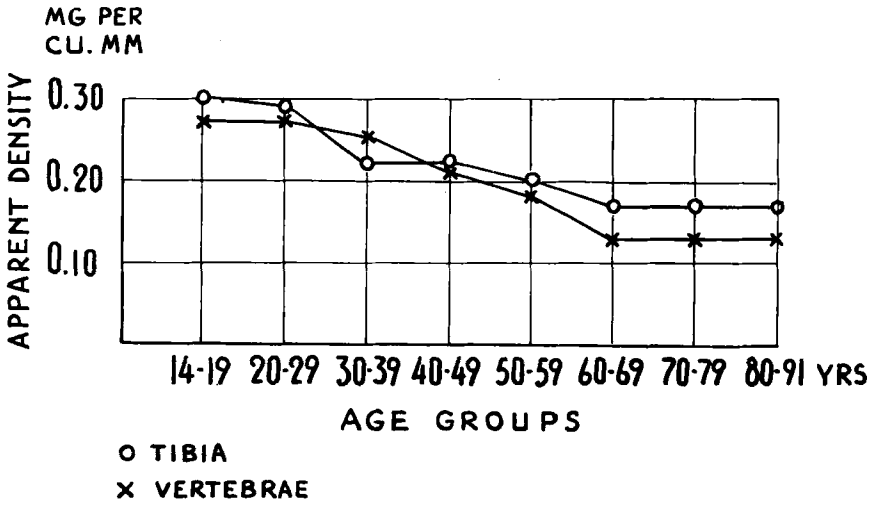


Fig. 7.
Variation of mean apparent density of vertebrae and tibia with respect to age group. Values for men and women combined.

Variation with age.—The maximum mean apparent density was found in the 14–19 year age group; there was then a gradual decrease until 60–69 years, after which the values remained constant. The variation was parallel for the tibia and vertebrae up to the 50–59 year group, after which the mean values for the vertebrae were slightly lower. Analysis of the individual values for the vertebrae from subjects below 60 years (Fig. 8) showed a negative, and highly significant, regression for apparent density on age ($P<0.001$). The same trend was found for the tibia (Fig. 9), except that in the case of the subjects above 60 years the values for these specimens were slightly higher than those for the vertebrae. Here, too, the negative regression was highly significant. For subjects above 60 years the means were significantly higher for the tibia than for the vertebrae ($P<0.001$), but there was a large variation between individuals (Figs. 8 and 9).

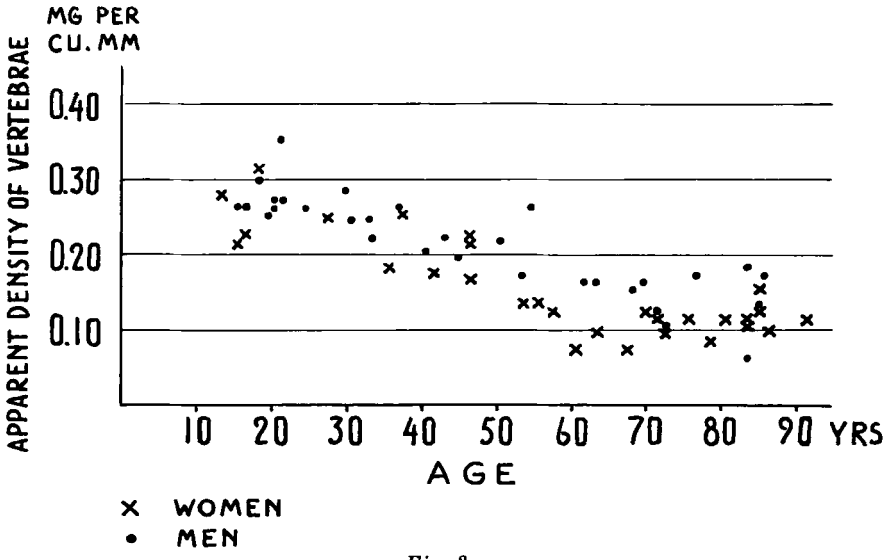


Fig. 8.
Relationship between age and apparent density of the vertebrae.

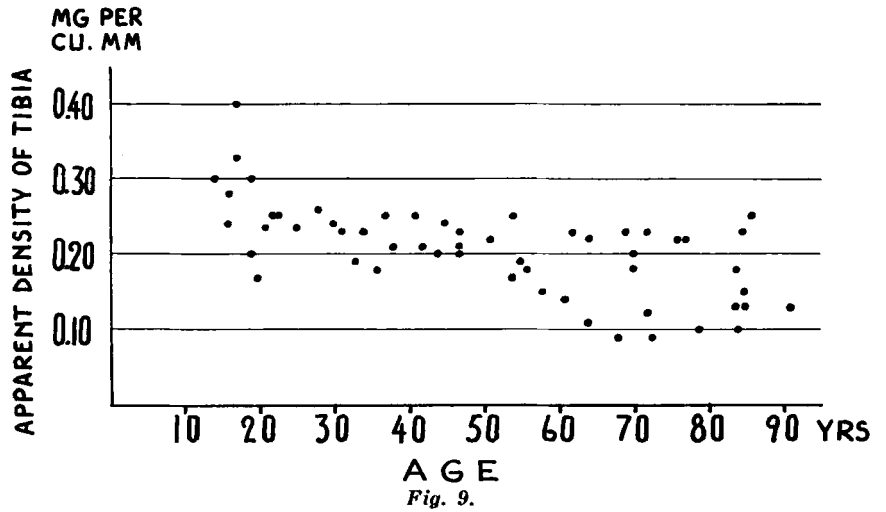


Fig. 9.
Relationship between age and apparent density of the tibia.
Each dot represents one subject.

Variation between the sexes.—The decrease in apparent density of the vertebrae was parallel in the sexes up to 60 years, with the means for the women consistently lower than those for the men (Fig. 10). The values for the men were in general higher than those for the women of

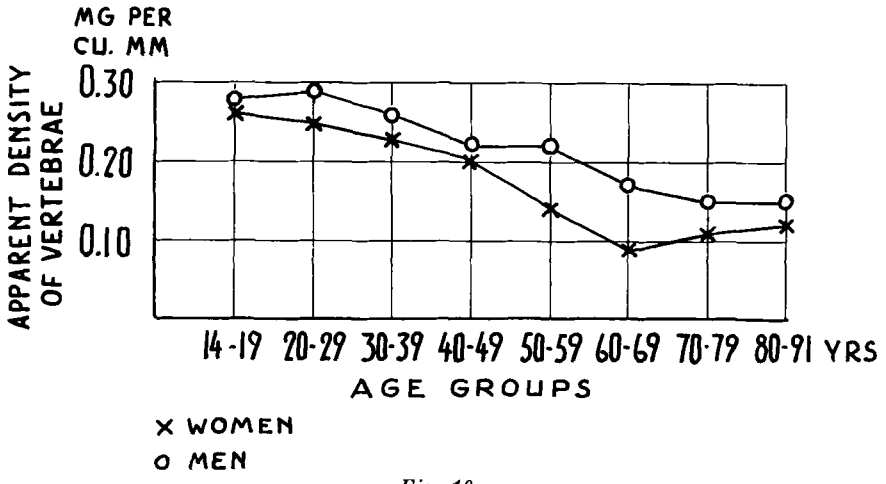


Fig. 10.
Variation of mean apparent density of the vertebrae with respect to age group.

the same age. In none of the groups was the difference significant, but it was so when all the age groups were combined ($P<0.001$). The values for the vertebrae may be considered more representative than those for the tibia, since each value is a mean of those for several vertebrae.

DISCUSSION

The gross evaluation of osteoporosis at autopsy would appear to give a fairly reliable grading in cases where the porosity is marked, but the method is not precise enough for distinguishing mild grades of the condition. Nor does microscopic examination, with determination of the relative area of bone, provide an exact method for grading the porosity, for although it is acceptable for a grouping on the basis of the means of many specimens, in the individual case it can give misleading results. It is therefore of doubtful value for estimating the degree of osteoporosis for small specimens. The same conclusion was yielded by an earlier study by *Lindahl* on the basis of microscopic examination of some 70 autopsy specimens of osteoporotic bone (9). *Ball* (4), who advanced the same opinion, maintained that there was no microscopic standard for classifying the thickness of trabeculae and the size of the spaces.

The results of this study confirm the supposition that the determination of the apparent density of spongy bone is the most accurate method for finding the average porosity of spongy bone. It has been asserted that in osteoporosis only the spinal column and the pelvis are

involved, the bone in the extremities remaining unaltered (2). The present study, however, showed a gradual increase in porosity with age for both vertebrae and tibia, though the effect at advanced age was more marked in the vertebrae. It seems to be generally held that osteoporosis is a disease that attacks certain persons, while the vast majority are unaffected by it. This view is not borne out, however, by the gradual reduction in the porosity of the tibia and vertebrae with age, from 20 years upwards, observed in this study; in fact, it would seem that the weakening of the spongy bone with age is, on an average, a more or less normal process. The fact that this process has not been noted earlier is attributable to the absence hitherto of any accurate method of gradation; for radiography does not provide a reliable diagnosis until the process is rather advanced. Moreover, the discovery of the disease by radiography is, of course dependent on the patient's consulting a physician. In view of the findings of this investigation, the considerably higher incidence of osteoporosis among women that is reported in the literature may not, as is commonly held, be due to deficient excretion of female hormones after the climacteric. Since the apparent density has been shown to be consistently lower for women than for men, a diagnosis of osteoporosis must have been made more frequently, and earlier, for women by a method that does not disclose the disease until it is fairly advanced. A division of cases of osteoporosis into post-climacteric and senile implies a correlation with time rather than with cause, and such a classification is not supported by the results of this study.

To examine the validity of the widelyheld view that muscular disuse can give rise to osteoporosis, autopsy specimens were obtained from subjects that had been confined to bed for long periods owing to neurologic disorders. In three cases where the period was more than 5 years the apparent density was extraordinarily low:

		Apparent density (mg/mm³)	
	Age	Tibia	Vertebrae
Case 1	29	0.10	0.11
Case 2	32	0.13	0.13
Case 3	43	0.11	0.13

The gradual development of osteoporosis from the age of 20 years, the more marked porosity among women, the large individual variation and the severity of the condition after long muscular disuse in-

dicate that inactivity is an important factor in the development of osteoporosis. In practice there will, of course, be other contributory factors such as depletion of calcium and cortisone therapy.

DEFINITION OF OSTEOPOROSIS

There is a certain variation among the definitions of osteoporosis, but common to all is the view that this condition includes a deficiency of bone substance owing to defective formation of matrix. The ash content, calcium content and bone density are normal (1, 11).

Any definition of osteoporosis should enable one to decide whether or not the condition is present; it would seem that none of the current definitions satisfies this requirement. Theoretically, defective matrix formation may be regarded as a definition of osteoporosis; in practice, however, it is difficult to ascertain whether there is such a deficiency, for there are no normal values for the content of bone matrix in the body, nor are there methods of ascertaining this quantity in any particular case. The problem is, in fact, difficult to solve. Since the true density of osteoporotic bone is the same as that of normal bone, it should be possible to determine the amount of bone matrix from the total weight of the whole skeleton, exclusive of soft tissues. This must be divided by the weight of the body. The quotient then obtained is rather unreliable, owing to the different proportions of fat and muscle.

The statement included in most definitions of osteoporosis that the disease is due to defective formation of the bone matrix is open to criticism, for this is no more than a hypothesis. Such statements tend to set development on wrong paths, since we are at present unaware of the cause of osteoporosis. Of the four theories that are most widely advanced—disturbance of protein metabolism, chronic deficiency of calcium, muscular disuse and abnormally low secretion of oestrogens—only the first is consistent with this definition-cum-hypothesis.

The mean apparent density of the tibia specimens of the 14–29 year groups, which may be regarded as largely normal, was 0.28 mg per cu. mm for 5 women and 0.31 for 9 men, with a mean of 0.30 for the whole group of 14 subjects. According to the available information, all these patients had been healthy, and death was due to sudden external violence, poisoning or acute illness. The bone was both grossly and microscopically normal. The variation in the apparent density for these cases was large (0.17, 0.20, 0.22, 0.24, 0.24, 0.25, 0.26, 0.28, 0.28, 0.30, 0.30, 0.33, 0.40, 0.76), but most of the values lay within the the range 0.20–

0.30. Variations within the normal limits would be due to, for instance, differences in physical activity. The extreme value 0.76 was for a powerfully built, 22-year-old man.

For the 11 tibia specimens that were classed by gross examination as severely osteoporotic, the mean apparent density was 0.12 mg per cu. mm (range 0.09–0.18). The corresponding figure for these 15 vertebrae specimens was 0.11 mg per cu. mm (range 0.07–0.19). On the basis of these figures, it is reasonable to define osteoporosis as a decrease to below 0.20 mg per cu. mm in the apparent density of the spongy bone in the upper end of the tibia, provided that microscopic examination discloses no pathologic condition of the bone.

Essentially the same definition might apply to the spongy bone of the vertebrae, but it is not so easy to obtain specimens from this region in the living subjects.

In one case where a value of 0.07 was recorded for a vertebra specimen, and in others with values of about 0.10 it appeared from the gross examination that further porosity would result in compression of the vertebral bone so that its apparent density would then increase again.

An apparent density of, for instance, 0.15 mg per cu. mm might not seem low enough to deserve attention, but when converted to a percentage, with 0.19 as 100 per cent and 0.09 as 0 per cent, this value is seen to correspond to 60 per cent of the normal porosity—a figure that, perhaps, gives a more realistic impression of the degree of osteoporosis.

SUMMARY

The apparent density of spongy bone in osteoporosis would seem to provide an exact measure of the degree of osteoporosis. To examine the validity of this supposition a study was made of autopsy specimens of tibia and vertebrae obtained from 60 subjects of ages ranging from 14 to 91 years. Beside gross and microscopic evaluations, the relative bone area was determined in sections prepared from the specimens. None of these methods was found to be accurate enough to permit of a reliable grading of osteoporosis in the individual case.

The average apparent density—or weight of unit volume—of spongy bone for various age classes ranged from 0.33 to 0.11 mg per cu. mm, and extreme individual values of 0.76 and 0.07 were recorded. The mean apparent density was a maximum in the 14–19 year age group and decreased steadily to 60–70 years. The variation was largely parallel for the tibia and vertebrae specimens. The apparent density was significant-

ly lower for women. The reduction with age was essentially the same for men and women.

On the basis of these results it would seem reasonable to define osteoporosis as a reduction in the apparent density of the tibia to below 0.20, provided that the microscopic examination discloses no pathologic alterations.

RESUME

La densité apparente de l'os dans l'ostéoporose pourrait sembler fournir une mesure exacte du degré d'ostéoporose. Afin de contrôler la validité de cette supposition, une étude a été faite en procédant à l'autopsie de spécimens de tibia et de vertèbre provenant de 60 sujets dont l'âge variait entre 14 et 91 ans. En plus des examens d'ensemble et microscopiques, la surface relative des os a été déterminée en préparant des sections des spécimens.

Aucune de ces méthodes n'a été considérée comme suffisamment exacte pour permettre d'évaluer le juste degré d'ostéoporose dans un cas individuel.

La densité moyenne apparante – ou le poids par unité de volume – du tissu spongieux dans différentes classes d'âges varie entre 0,33 et 0,11 mg par mm^3 et les valeurs individuelles extrêmes de 0,76 à 0,07 ont été enregistrées. La densité moyenne apparente atteint un maximum dans le groupe d'âge entre 14 et 19 cas pour décroître constamment jusqu'à 60–70 ans. La variation est largement parallèle pour les spécimens de tibia et de vertèbre. La densité apparente est nettement plus basse chez les femmes. La réduction de la densité avec l'âge est essentiellement la même chez les hommes que chez les femmes.

Sur la base de ces résultats, il paraît raisonnable de définir l'ostéoporose comme la réduction de la densité apparente du tibia à moins de 0,20, à condition que l'examen microscopique ne révèle pas d'altérations pathologiques.

ZUSAMMENFASSSUNG

Die augenscheinliche Dichte des Knochens bei der Osteoporose scheint einen genauen Masstab für den Grad der Osteoporose zu ergeben. Um die Gültigkeit dieser Vermutung festzustellen wurde eine Untersuchung an Autopsiepräparaten von Tibia und Wirbeln, die von 60 Individuen im Alter von 14 bis 91 Jahren stammten, vorgenommen. Abgesehen von grober und mikroskopischer Bewertung, wurde die relative Knochenfläche in Schnitten, die von den Präparaten zubereitet waren, bestimmt.

Keine dieser Methoden erwies sich genau genug, um eine verlässliche Schätzung der Osteoporose im einzelnen Falle zu gestatten. Die durchschnittliche, scheinbare Dichte – oder Gewicht des Einheitsvolumen – für die Spongiosa verschiedener Altersklassen reichte von 0,33 bis 0,11 mg/mm^3, und extreme individuelle Werte von 0,76 bis 0,07 wurden verzeichnet. Die scheinbare Durchschnittsdichte zeigte ein Maximum in der 14–19 Jahre Altersgruppe und nahm fortlaufend bis zu 60–70 Jahren ab. Die Verschiedenheit lief im Grossen und Ganzen parallel in den Tibia- und Wirbelpräparaten. Die augenscheinliche Dichte war deutlich niedrige beim weiblichen Geschlecht. Die Herabsetzung mit dem Alter war im wesentlichen die gleiche bei Frauen und Männern.

Auf Grund dieser Ergebnisse würde es angebracht Osteoporose als eine Verminderung der scheinbaren Dichte der Tibia unter 0,20 zu definieren, vorausgesetzt, dass die mikroskopische Untersuchung keine pathologischen Veränderungen aufzeigt.

REFERENCES

1. *Agna, J., Knowles, H. & Alverson, P.:* J. Clin. Invest. *37*: 1357–1361, 1958.
2. *Albright, F. & Reifenstein, E.:* The Parathyroid Glands and Metabolic Bone Disease. The Williams & Wilkins Company, Baltimore, 1948, pp. 145–204.
3. *Ardran, G.:* Brit. J. Radiol. *24*: 107–115, 1951.
4. *Ball, J.:* Diseases of Bone. Recent Advances in Pathology. Edited by C. V. Harrison. Ed. 7. J. & A. Churchill, Ltd., London, 1960.
5. *Broman, G., Trotter, M. & Peterson, R.:* Am. J. Phys. Antropol. *16*: 197–211, 1958.
6. *Fusi, G.:* Radiol. Clin. *22*: 123–131, 1953.
7. *Lachman, A.:* Radiology *26*: 165–177, 1936.
8. *Landorff, G. A.:* Acta chir. Scand. Suppl. *71*: 1942.
9. *Lindahl, O.:* Acta orthop. Scand. *29*: 160–163, 1960.
10. *Lindahl, O.:* Acta physiol. Scand. Suppl. 179, *51*: 1961.
11. *Rutinhauser, E. & Maulbetsch, A.:* Beitr. z. path. Anat. u. z. allg. Path. *94*: 332–344, 1934.
12. *Sante, L. R.:* Principles of Roentgenological Interpretation. Revised ed. 10. Edwards Brothers Inc., Michigan, 1955, p. 552.