Clinical Orthopedics + Related Research V. 78
REPOSITORY MMES-ORNL
COLLECTION Snyder's Files
BOX No. 3
FOLDER Misc.
724017

Quantitative Characterization of Bone: A Computer Analysis of Microradiographs

ELIZABETH LLOYD* AND D. HODGES†

An automatic scanning device under the control of a computer (CHLOE)[15] has been used to analyze microradiographs from a large number of bone sections from man, dog, and mouse. Our previous publications have dealt only with surface/volume ratios for a very small number of bones. The present report is intended to provide a comprehensive survey of all of the parameters measured for a much larger number of bones. These parameters include bone volume, bone surface, trabecular thickness, and sizes of marrow cavities.

All of these results can be obtained in less than a minute per sample. A comparison is made between values for the human skeleton and those for dogs and mice. The amount of marrow irradiated by a radioisotope deposited in bone will depend on the size of the marrow cavities, and the endosteal dose to bone will depend on the surface/volume ratio. Some isotopes deposit throughout the whole volume of bone (such as ^{226}Ra), while others deposit on bone surfaces (such as ^{239}Pu or ^{241}Am). If we consider the endosteal surface and the marrow to be the target tissues for irradiation damage, then the relative biological effectiveness of these isotopes may be different in man and dog if the surface/volume ratios of bone and the percentage of marrow irradiated are different. (The relative rates of bone turnover may also be an important factor.)

In addition to the problem of relative toxicity of radioelements, measurements of the microscopic structure of bone are important for the diagnosis of bone diseases. In studying small biopsy samples of bone it is important to know the variation in structure in different bones in the same normal individual as well as the type of variations to be expected due to small differences in positioning of the exact sampling location.

For our study we obtained all the bones of a human skeleton of an Asian male in his mid-forties. The total skeletal, dry, fat-free weight was 3.6 kg. Table 1 shows the relative weights of the individual bones. For comparison the table also gives values for the average values of 10 adult male skeletons described by Trotter and Peterson.[22] As we can see from the table, the relative values for the weight of different bones of the skeleton are very similar to the normal average values. From these data, together with the fact that no abnormalities were observable in the microradiographs, it was concluded that the skeleton was morphologically normal skeletally. We also measured bone for 3 dogs and 1 mouse.

This work was performed under the auspices of the U. S. Atomic Energy Commission, Radiological Physics Division, Argonne National Laboratory, Argonne, Illinois 60439.

* Radiological Physics Division, Argonne National Laboratory.

† Applied Mathematics Division, Argonne National Laboratory.

A-00617
Human Studies Project
1169816

METHODS AND MATERIALS

Individual bones from the adult skeleton were cut into convenient sizes, usually about 5 cm in length, for embedding in methyl methacrylate. Sections 100 μ thick, and less than 1 inch square, were cut from these pieces. The sections were x-rayed using a Machlett x-ray tube with a tungsten filament, operated at 5 KeV and 20 mA. The section was mounted in close contact with a high contrast film (Eastman Kodak, fine grain, positive). This was loaded into a 35 mm camera attached to the exit port of the x-ray tube. A typical exposure for a 100 μ thick section was 4 minutes. The film was developed in a high contrast developer (dextrol, 1:1 dilution) for 1½ minutes.

The 35 mm film used here had several advantages over more conventional spectroscopic plates. First, scanning of the film to determine the perimeter and area was greatly facilitated by obtaining maximum contrast between the bone (white areas) and those areas where bone was absent (black areas). Second, 35 mm film made possible the automatic scanning of a large number of pictures on a roll of film; the computer program provided for film advance under the control of an on-line typewriter.

SCANNER

The automatic scanner (CHLOE) was designed to digitize data from photographic film. A detailed description of its design and operation is given elsewhere[9, 10]; for the sake of completeness, a very brief summary of the operation is included here.

A spot of light (about 25 μ in diameter) generated by a cathode ray tube is focused onto the film, and the transmitted light is detected by a photomultiplier. The light spot moves in discerete steps in the x-direction and then returns to make successive scans. The separation of these scans can be varied by factors of 2 down to 7 μ. All parameters of the scan, including the rectangular boundaries of the area scanned, are under the control of the on-line typewriter. The scanner system records the coordinates of the point at which the light spot detects a change from black to white or from white to black. A pair of such points marks the beginning and end of a line segment parallel to the x-axis. The equipment can be programmed so that the line segments lie over either white areas on a black background or black areas on a white background.

TABLE 1. Relative Weights of Individual Bones Taken from the Whole Skeleton
(values expressed as the percentage of the whole skeletal weight)

	Trotter and Peterson[22] *(Av. 10 adult skeletons)*	*Lloyd (1 skeleton)*
Skull	17.1	18.9
Femora, tibiae, fibulae	32.0	31.0
Humeri, radii, ulna	11.9	10.0
Vertebrae	10.5	13.2
Ribs	6.3	6.3
Other bones (including hands, feet, and innominate bones)	22.2	20.6
	100.0	100.0
Total skeletal weight	3.86 kg	3.59 kg

AREA

The area of bone is measured by taking the sum of the lengths of all line segments lying over the white area of a microradiograph and multiplying by the vertical separation between scans. Figure 1 shows the line segments over a small portion of trabecular bone. The line segment length in each linear scan is obtained by subtracting the x-coordinates of the beginning and end of each line segment when scanning in the x-direction. These are summed for all of the line scans—up to 2^{12} (4096) per inch. The scanning area is calibrated by using a 2-mm grid on 35 mm film and counting the number of scanning lines in each of the x- and y-directions corresponding to a known grid size.

PERIMETER

The perimeter of bone, P, was determined by summing the distances between the adjacent pair of end points where successive line scans intersect the bone (square root of the sum of squares of the differences in x- and y-coordinates). This does not involve the assumption of random shapes as does the formula

1169817

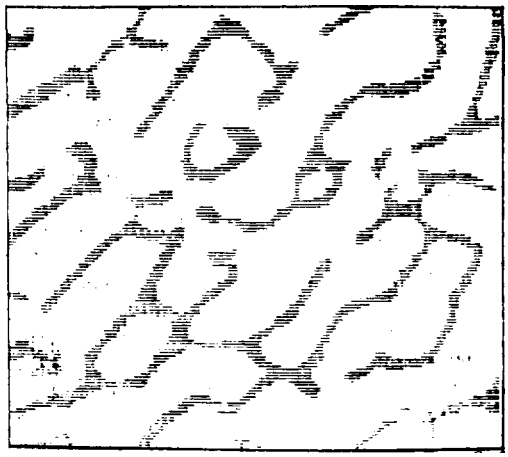

FIG. 1. Computer print-out of line segments over a small portion of trabecular bone.

TABLE 2. Typewriter Print-out from the Computer of the Values Obtained from the Automatic Scan of a Microradiograph of the Rib (the symbols used are identified below the print-out)

H CS*10 75 × 12 Y 12 I 913WOB RR6G22 164

NL 6679

LL/8 31629

P 015.729 cm

A 0.14233cm.cm

TA 1.79712 cm.cm

FA 0.07920

ALS 284 microns

P/A 110.513 cm/cm.cm

The first line refers to the conditions of measurement and the picture designation.

NL = number of line segments
LL = length of line segments
P = perimeter
A = area
TA = total area scanned
FA = fractional area over bone
ALS = average line segment length
P/A = perimeter divided by area

of Henning[8] based on the number of line segments and used in previous work, but is an accurate measure of perimeter no matter what shape is measured.

SURFACE/VOLUME RATIOS

When cortical bone is measured, the ratio for surface/volume is identical to the two-dimensional ratio of perimeter/area because the bone surfaces in a cross section are not randomly oriented but lie predominantly parallel to the long axis of the bone. For trabecular bone, however, the orientation is more random and the difference between the two-dimensional and the three-dimensional ratio requires a correction of $4/\pi$. This factor and the reason for its use is discussed fully in the Appendix. This means that the values reported in our earlier paper for perimeter/area have to be increased by a factor of 1.273 to give the correct values for the surface/volume ratios for trabecular bone, although the surface/volume values for cortical bone are the same as those reported for perimeter/area. Table 2 shows a typical typewrite print-out from the computer for an automatic scan of a bone section (rib).

RESULTS

FEMUR—CORTEX

The femur was segmented as shown in Figure 2. Figure 3 shows Haversian canals and resorption cavities seen in the microradiograph of cortical bone taken from segment 4 (Fig. 2). Figure 4A is an enlargement of the "boxed" area of bone indicated in Figure 3, and Figure 4B is a computer print-out of the line segments generated over the holes seen in the microradiograph. The separate line segments are not visible because the line spacings are only 7μ apart. Table 3 gives values for the perimeter, area of bone studied, and surface/volume ratios for four different areas of the cortex. As already mentioned, the surface/volume ratio is numerically equal to perimeter/area for cortical bone. These measurements do not include the periosteal and endosteal surfaces. Separate measurements indicate that these surfaces add about 10 per cent to the values listed.

1169818

Head of Femur

Figure 5 shows the microradiograph (5A) and the computer print-out (5B) of a section taken from segment 1 of Figure 2. Table 4 gives values obtained for 8 sites taken from this bone segment. As previously mentioned, and discussed later, the surface/volume ratio is obtained by multiplying the perimeter/area by 1.273.

Vertebra

Figure 6 shows a thin section taken from the seventh thoracic vertebra. The "boxed in" area indicates the region over which the measurements were made. Values are given in Table 5 for the perimeter, area, and surface/volume ratios for the "boxed in" portions of bone taken from 8 different sections. The effect of including the whole bone is also included in Table 5 for the same sections. When the cortex of the bone is included, the surface/volume ratios are reduced by about 5 per cent from the values obtained for pure trabecular bone. Average values for the length of the line segments over bone are given in Table 6, both for the horizontal and vertical directions of scan. These values refer to pure trabecular bone. When the scan was in the horizontal direction, the average length of the line segments was about 25 per cent less than when it was in the vertical direction because the trabeculae were primarily parallel to the vertical direction of the scan. The average length for the line segments in the horizontal direction was about 300 μ. However, the variations in average line segment lengths for the 8 sections was about a factor of two (200–400 μ).

Table 7 gives the corresponding values made over the marrow cavities. Again, as expected, the horizontal scans show average line segment lengths about 25 per cent less than those found in the vertical scans. These values ($\sim$ 800 μ) are about two to three times the values obtained for the segment lengths over the bone, which fits well with a fractional volume of bone in any section

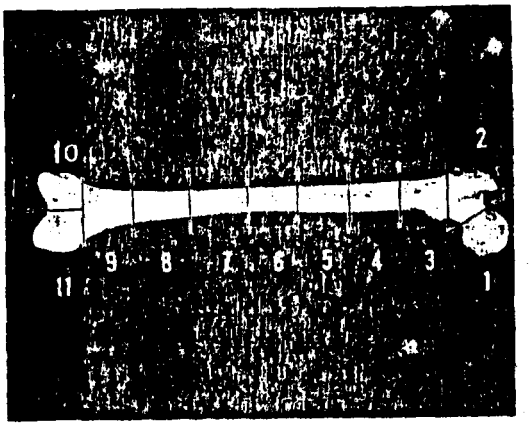

FIG. 2. Human femur showing the 11 segments into which the bone was cut for sampling.

being about 25 per cent of the total volume. Exact measurements of fractional bone volume were made for the third lumbar vertebrae. These are given in Table 8 and show variations between 20 per cent and 36 per cent for serial sagittal sections. Values for perimeter, area, and surface/volume ratios for the third lumbar vertebrae are also given in Table 9. The surface/volume ratios appear to be of the same order as those found for the thoracic vertebra, although they are about 15 per cent higher than those for the femoral head (Table 4). Average values for the line segment length over bone are given

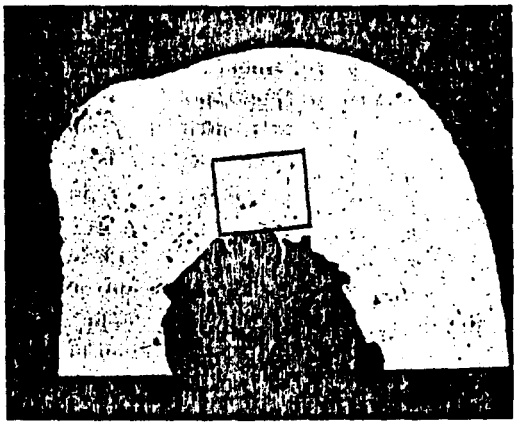

FIG. 3. Microradiograph of a cross-section of bone taken from the mid-cortex of the femur.

1169819

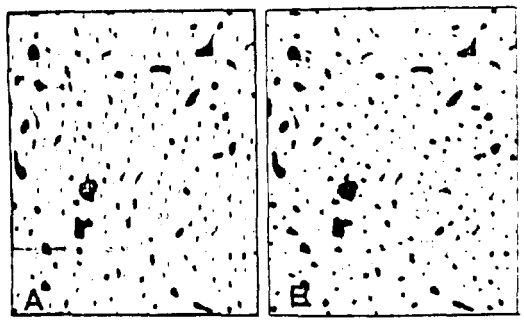

FIG. 4 A–B. A. Microradiograph, at a higher magnification, of the portion of bone indicated by the square in Figure 3. B. Corresponding print-out of the same portion of bone.

in Table 10. The average value for the horizontal measurements is 300 μ. This is very close to the value of 296 μ found for the thoracic vertebra (Table 6).

RIB

The sixth rib was first cut into 7 segments along its length, each segment about 4.5 cm long. Measurements of 40 different cross-sections were made.

Figure 7 demonstrates the appearance of microradiographs of the cross-sections of bone taken at the junction of each of the 7 segments. It is interesting to note that close to the costal cartilage junction the cortex is very thin and this encloses a large number of very fine trabeculations. About 5 cm from the vertebral end, the cortex is much thicker and the trabeculations are fewer and coarser. Table 11 gives values for the cross-sectional area, together with the surface length or perimeter of each of the bone sections studied. Values of perimeter/area and surface/volume ratios are also listed. A correction factor of 1.13 was applied to convert from perimeter/area to surface/volume instead of the 1.27 used for pure trabecular bone because about ½ of the bone was cortical.

The sections were numbered, starting with number 1 taken from the sternal end of the rib ranging up to number 72 taken from the vertebral end (Table 11). The bone area changes from 0.10 cm² to 0.36 cm² while the perimeter changes from 12.9 cm to 29 cm. The bone area is greatest about 5 cm from the vertebral end where the cortex is thickest. The perimeter is small at this point, giving a value of 43 cm/cm² for perimeter/area (P/A) compared with a value about 5 times greater obtained for a section taken close to the sternal end. The mean marrow space taken from the middle of the rib measured 1200 μ, and the width of the trabeculae at this site averaged about 100 μ.

TABLE 3. Perimeter, Area, and Surface/Volume Ratios of Cortical Bone from the Midshaft of the Femur

Section Number	*Perimeter (cm)*	*Area (cm²)*	*Surface/Volume (cm²/cm³)*
RF 1	7.6	0.23	34
RF 2	9.6	0.32	31
RF 3	8.2	0.30	27
RF 4	4.0	0.13	30
		Mean	30

Note: (Perimeter/Area) is numerically equal to (Surface/Volume)—see text.

ILIAC CREST

Figure 8 illustrates the cuts which were made to study the iliac crest. Examples of microradiographs taken from sections along its length are also shown. Table 12 gives values for pure trabecular bone together with values obtained for the whole bone section. Values for the whole bone are about 30 per cent lower than those for the central portion of pure trabecular bone. The percentage volume of bone is very similar to that obtained for the third lumbar vertebra (about 25%). The mean value for the trabecular thickness was 150 μ, and the average segment length of the marrow spaces was

1169820

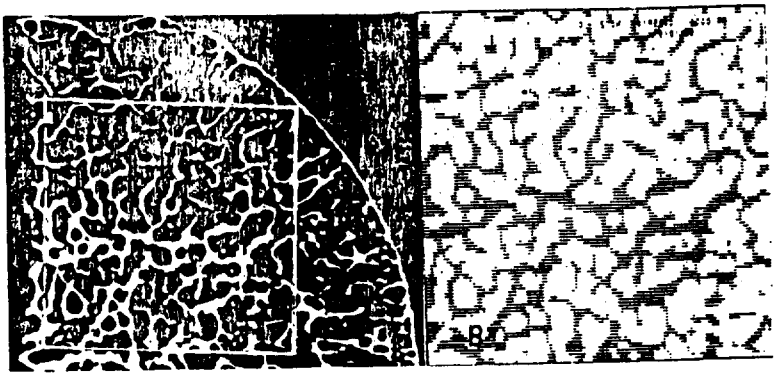

FIG. 5 A–B. A. Microradiograph of a portion of bone taken from the head of the femur. B. Computer print-out of the same portion of bone.

about 800 μ in a section taken about 2 cm from the anterior superior iliac spine. These values are very similar to those obtained for the thoracic vertebra, except that the trabeculae are somewhat thinner.

Cranium

Sections of the cranium were studied at 2-cm intervals starting at the bregma and moving posteriorly parallel to the coronal suture as shown in Figure 9. Typical microradiographs taken at different sites are also illustrated. Table 13 gives values for the perimeter and area together with values for the surface/volume ratio. The correction factor of 1.13 was used to convert from perimeter/area to surface/volume. The average value for surface/volume ratio of the 9 sections measured was 41 cm^2/cm^3. This value is somewhat greater than the value for cortical bone from the midshaft of the femur, but about a factor of 3 less than the trabecular bone of the iliac crest.

Measurements on Beagle Dogs

Lumbar vertebra. The lumbar vertebra of 3 beagle dogs (kindly given us by Dr. Webster Jee, University of Utah), two aged 2 years (MAC 56 and MAC 58) and one aged 3 years (MAC 71) were sectioned sagitally in the same way as the human lumbar vertebrae. Figure 10 shows sagittal sections from the vertebrae of a man, a dog, and a mouse at the same magnification. Values for the surface/volume ratios for two sections from each dog are given in Table 15. The percentage volume of bone for the whole bone section and for a "boxed in" portion of trabecular bone, excluding the cortex, are also given. The values for the surface/volume ratios (about 200 cm^2/cm^3) are 50 to 100 per cent higher than the values

TABLE 4. Perimeter, Area, and Surface/Volume Ratios of Trabecular Bone from Head of Femur (Segment 1)

Section Number	*Perimeter (cm)*	*Area (cm^2)*	*Perimeter/Area (cm/cm^2)*	*Surface/Volume (cm^2/cm^3)*
Ba 5	48	0.65	74	94
Ba 7	47	0.58	81	103
Ba 10	28	0.34	81	103
Ba 13	11	0.15	70	89
Bb 4	51	0.70	73	93
Bb 7	44	0.63	70	89
Bb 9	37	0.46	79	100
Bb 12	23	0.30	77	98
		Mean	76	96

Note: (Surface/Volume) is calculated by multiplying (Perimeter/Area) by 1.273—see text.

1169821

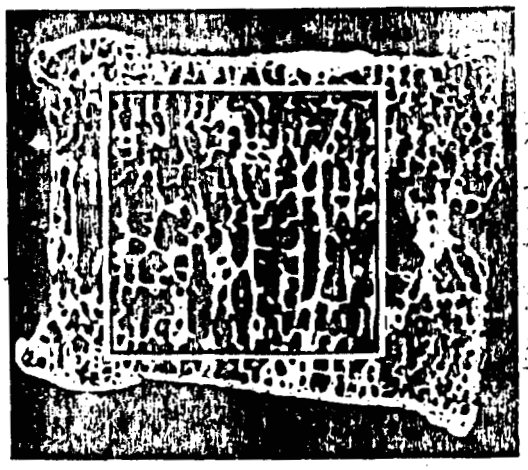

FIG. 6. Microradiograph of the seventh thoracic vertebrae.

obtained for the human; values of the percentage bone volume within the cortex (about 40%) are also greater by about the same amount. Correspondingly, values for the average line segment lengths over the marrow spaces (300 μ) are much less than in the human vertebrae (about 800 μ). In the dog, average values for line segment lengths over bone and over the marrow spaces are of the same order (Table 15), unlike the situation in humans where the segment lengths over the marrow spaces are in general two or three times the segment lengths over the bone.

MEASUREMENTS ON MICE

Lumbar vertebra. So far, the measurements on mice (Fig. 10) have been confined to a very few sections; however, even for these few sections it is obvious that the surface/volume ratios are even larger than for the dog. Average values range between 250 and 350 cm^2/cm^3. The trabecular thickness is much smaller, in the region of 80 μ for an adult mouse (5 months old).

Femur. Provisional measurements on the femur cortex of mouse suggest that the surface/volume ratio is about 30 to 40 cm^2/cm^3. This value appears to be about the same as in the dog and in man.

DISCUSSION

These measurements have immediate application in the estimation of:

1. Quantitative structural differences between bones of the same skeleton.
2. Relative bone turnover rates in different regions of the same skeleton.
3. Radiation doses from bone-seeking isotopes.
4. The fraction of the total bone surface in cortical and in trabecular bone.
5. The relative biological effectiveness of surface and volume bone seekers in animals and in man.
6. Exchangeable bone calcium.

There is no doubt that many other applications of these measured parameters will be made, perhaps in areas we have not foreseen. For this reason, we have tabulated our data in full for others to use in their own calculations.

1. Quantitative Structural Differences Between Bones. In the human skeleton, cortical bone taken from different sites appears to show very little difference in structure. More work, however, is needed to provide a realistic range of values to cover the extent of the variation found between different cortical bones. It is interesting that the values obtained for the surface/volume ratio of cortical bone in both dog and mouse bone are of the same order as those found in human cortical bone (30 cm^2/cm^3).

For trabecular bone, as far as is known, there are no published data on the quantitative structural differences of different cancellous bones taken from the same skeleton. Table 16 was therefore compiled to show typical values for four different trabecular bone sites. Perhaps the most significant feature in this comparison is the similarity in the average values for each parameter for different trabecular bones even though, as seen in the preceding tables, there is a wide variation between samples taken from the same bone. The percentage volume of the bone within the cortex of the iliac crest, lum-

1169822

bar and thoracic vertebrae, and the head of the femur are all of the same order, between 25 and 30 per cent. The surface/volume ratios fall within the range of a factor of two (100–180). The average widths of the line segments over trabeculae also fall within a factor of two (220μ–330μ for the horizontal scans across the bone and 290μ–400μ for the vertical scans along the bone axis).

Although no published data are available on comparisons between different cancellous bones in the same individual, much published data are available on the physical characteristics of the lumbar vertebrae. Some of these studies have involved sampling of a large number of vertebrae from different postmortem samples to determine changes with age, sex, and disease.[2-4] Table 17 summarizes the values obtained by other workers together with the values obtained in the present study. For purposes of comparison with our studies, only those normal cases in the 30–50 year age group were selected. The number of vertebrae studied and the thickness of the sections used are given in the table.

From the table we see that values for the percentage bone volume reported by all the authors for normal human lumbar vertebrae lie between 13.5 per cent and 33 per cent, except for one value of 69 per cent calculated from the porosity values given by Atkinson.[8] This high value may be the result of the method of measurement which involved using radiographs of very thick sections (2.5 mm. thick). It would appear that projection of the thickness of the section would lead to an overestimate of values for bone volume. In our work we were unable to demonstrate any difference in our bone volume measurements between sections which were 30μ and 100μ thick. The preparation of much thinner sections, it was felt, carried the risk of cracking or total loss of some spicules of bone. Our value of 25 per cent for bone volume would therefore appear to lie within the normal range of values reported by other authors.

TABLE 5. Effect of Region Scanned on Values of Perimeter, Area, and Surface/Volume Ratios of Sagittal Sections of the Seventh Thoracic Vertebra

	Whole Portion				Trabecular Portion			
Section Number	*Perimeter (cm)*	*Area (cm^2)*	*Perimeter/Area (cm/cm^2)*	*Surface/Volume (cm^2/cm^3)*	*Perimeter (cm)*	*Area (cm^2)*	*Perimeter/Area (cm/cm^2)*	*Surface/Volume (cm^2/cm^3)*
TV 7-A120	124	1.60	77	99	46.5	0.57	82	104
A121	126	1.02	123	156	40.6	0.32	126	160
A122	126	1.58	80	102	45.7	0.52	88	112
A123	130	1.48	88	112	45.8	0.48	94	120
A124	129	1.43	91	116	45.7	0.43	105	134
A125	125	1.57	79	101	45.2	0.55	82	105
A126	111	1.52	73	93	42.7	0.51	84	107
A127	105	1.52	69	88	46.8	0.71	66	84
		Mean	85	108			91	116

Note: (Surface/Volume) is calculated by multiplying (Perimeter/Area) by 1.273—see text.

1169823

TABLE 6. Average Length of Line Segments (in microns) Over Bone in Human Thoracic Vertebrae

Section Number	*Vertical Scan*	*Horizontal Scan*
A 120	451	302
A 121	302	197
A 122	407	289
A 123	369	269
A 124	328	247
A 125	399	322
A 126	411	310
A 127	531	384
Mean	400	296

Values reported for surface/volume ratios of bone mineral in the vertebrae are also given in Table 17. Our value is in good agreement with the value given by Amstutz and Sissons[1] and that reported by Bromley, *et al.*,[3] but it is almost a factor of two lower than the value of Dyson, *et al.*[5] Values for the surface area of bone per unit of bone tissue volume are also given in the table. These values are obtained by multiplying the surface area/unit volume of bone mineral by the fraction of bone volume. Our value of 32 cm^2/cm^3 is within the range of the values reported by others (19–33 cm^2/cm^3). Measurements on the iliac crest have also been made on a large number of cases by Wakamatsu and Sissons[23] who report an average value of 23 per cent for the percentage of bone volume; this is in good agreement with our value of 25 per cent. However, no values are available in their publication for line segment lengths over bone and marrow; instead they have defined another parameter, "trabecular bone thickness," which will always be smaller than our measurements of bone segment lengths because it is measured at right angles to each trabecula rather than randomly as in the present work.

TABLE 7. Average Length of Line Segments (in microns) Over Marrow in Human Seventh Thoracic Vertebra

Section Number	*Vertical Scan*	*Horizontal Scan*
A 120	915	627
A 121	1223	843
A 122	938	677
A 123	965	695
A 124	960	743
A 125	875	699
A 126	983	763
A 127	784	579
Mean	955	703

Other bone sites for which values should be available soon include radius, ulna, phalanx, temporal bone, clavicle, sternum, and os calcis.

2. Relation of Bone Mineral (Surface/Volume) Ratios to Bone Turnover. If we know the percentage of bone surface which is involved in bone formation, then knowing the surface/volume ratio of bone mineral together with the linear apposition rate we can calculate the percentage bone turnover.

In the midshaft of the adult femur, Jowsey *et al.*[11] found that 3 per cent of the bone surface was involved in bone formation. If we assume a linear growth rate of 1μ (10^{-4} cm) per day,[3] together with our surface/volume ratio of 30 cm^2/cm^3, then the fractional volume, F, of cortical bone formed per day will be

$$F = \frac{3}{100} \times 30 \frac{cm^2}{cm^3} \times \frac{10^{-4}\ cm}{day}$$
$$= 9 \times 10^{-5}/day$$

i.e., 0.009%/day or 3.3%/year.

This value is in good agreement with meas-

1169824

urements reported by Frost[7] using tetracycline labeling. It is also in close agreement with values reported by Rowland[17] which were derived from long-term radium studies. An average value of 7.4% for the percentage of bone-forming surface in the iliac crest has also been reported.[11] Using this value together with our surface/volume ratio of 180 cm^2/cm^3 and 1μ/day linear apposition rate (a value found by Lee[13] to be the same as in cortical bone), we can calculate the fraction, F, of trabecular bone formed per day.

$$F = \frac{7.4}{100} \times \frac{180\ cm^2}{cm^3} \times \frac{10^{-4}\ cm}{day}$$

$$= 1.33 \times 10^{-3}/day$$

i.e., 0.133%/day or 48.6%/year.

No reliable results for trabecular bone turnover have been reported using tetracycline labeling; however, the relative rates of trabecular and cortical turnover can be inferred from radioactive tracer experiments. In a ^{45}Ca tracer experiment in rabbits we found the rate of bone turnover in the trabecular bone (vertebrae)[12] to be higher than in cortical bone by about a factor of 4.

Values obtained for the concentration of ^{226}Ra in different human bones from the MIT series,[6] and plotted as a function of time

TABLE 8. Percentage Volume of Bone Mineral in Trabecular Bone Tissue of the Human Third Lumbar Vertebra

Section Number	*% Bone Volume*
131	25.2
132	27.9
133	27.2
134	24.5
135	36.0
136	27.9
137	24.5
138	19.7
139	24.5
140	29.9
Mean	26.7

TABLE 9. Perimeter, Area, and Surface/Volume Ratios of Bone Mineral in the Human Third Lumbar Vertebra

Section Number	*Perimeter (cm)*	*Area (cm^2)*	*Perimeter/Area (cm/cm^2)*	*Surface/Volume (cm^2/cm^3)*
131	36.9	0.37	97	124
132	39.1	0.41	93	119
133	39.2	0.40	98	125
134	37.9	0.36	104	133
135	42.8	0.53	79	99
136	37.9	0.41	91	116
137	34.9	0.36	96	122
138	33.7	0.29	113	143
139	32.9	0.36	90	115
140	35.9	0.44	82	105
		Mean	94	120

Note: (Surface/Volume) is calculated by multiplying (Perimeter/Area) by 1.273—see text.

1169825

TABLE 10. Average Line Segment Length (in microns) Over Bone and Marrow in the Human Third Lumbar Vertebra

Section Number	Bone		Marrow	
	Vertical Scan	Horizontal Scan	Vertical Scan	Horizontal Scan
131	365	286	1027	756
132	374	299	925	697
133	358	287	925	702
134	345	265	1040	742
135	432	360	748	581
136	383	308	958	735
137	336	315	1034	864
138	325	240	1213	859
139	371	327	1087	882
140	480	317	1100	702
Mean	377	300	1006	752

after intake,[14] also suggest about the same difference in relative turnover rates between trabecular and cortical bone. Hence, one might except values of about 12 to 15 per cent/year for trabecular bone turnover compared to the value of about 50 per cent calculated here from the percentage bone surface involved in bone formation. This divergence could result from an accumulation of errors all tending to make the calculated value too high. Such a set of circumstances would arise if (1) the linear growth rate of 1μ/day were too high, (2) the surface taking part in bone formation were overestimated, or (3) the rate of turnover in the iliac crest were greater than the rate of turnover in the vertebrae. Measurements for the surface/volume ratio of the iliac crest reported here (180 cm^2/cm^3) appear to be in excellent agreement with those found by Sissons, *et al.*[19] (183 cm^2/cm^3 for cases aged 20–50). Hence, it is unlikely that the error in the calculation is due to an error in the surface/volume ratio.

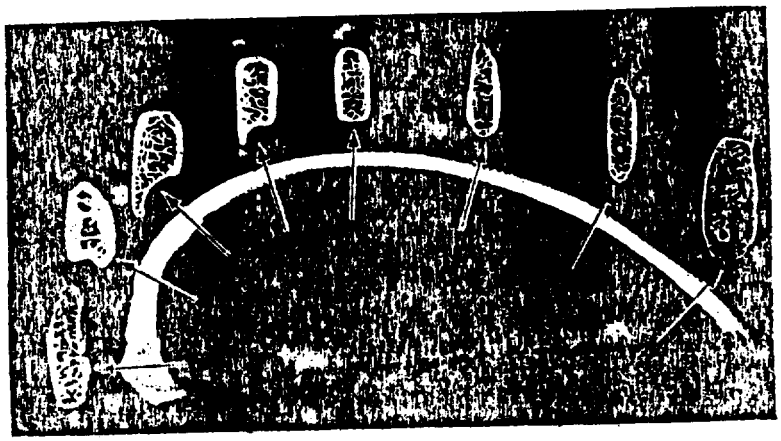

FIG. 7. Picture of the sixth rib showing the appearance of microradiographs made from thin sections of bone from different locations.

116982b

TABLE 11. Perimeter, Area, and Surface/Volume Ratios in Cross Sections of Bone Taken from Different Positions Along the Length of a Human Rib

	Section Number	Area Bone (cm^2)	Perimeter Bone (cm)	Perimeter/Area (cm/cm^2)	Surface/Volume (cm^2/cm^3)
Sternal end	8	0.16	28.0	176	195
	9	0.18	29.3	163	184
	10	0.13	25.1	193	218
	11	0.14	24.9	178	201
	13	0.11	19.4	176	199
	15	0.13			
	17	0.10	16.5	165	186
	19	0.13	15.2	117	132
	20	0.10	14.8	148	167
	21	0.12	15.2	127	144
	22	0.13	14.0	108	122
	25	0.13	17.3	135	153
	26	0.14	14.9	106	120
	28	0.14	15.1	107	121
	30	0.16	12.8	80	91
	31	0.16	13.1		
	34	0.16	14.7	92	104
	35	0.17	14.5	85	96
	38	0.16	15.1	94	107
	39	0.16	14.7	92	104
	42	0.18	14.8	82	93
	43	0.18	13.3	73	83
	45	0.22	17.1	77	88
	46	0.21	16.7	79	90
	49	0.20	16.4	82	93
	50	0.20	16.8	84	95
	52	0.23	20.0	87	98
	53	0.21	19.7	94	106
	55	0.24	19.8	82	93
	56	0.24	19.6	81	92
	60	0.36	15.5	43	48
	61	0.36	14.8	41	46
	63	0.34	12.9	38	43
	64	0.33	12.9	39	44
	66	0.27	14.8	55	62
	68	0.27	18.1	67	76
Vertebral end	72	0.25	21.0	84	95

Note: (Surface/Volume) is calculated by multiplying (Perimeter/Area) by 1.13—see text.

1169827

8286911

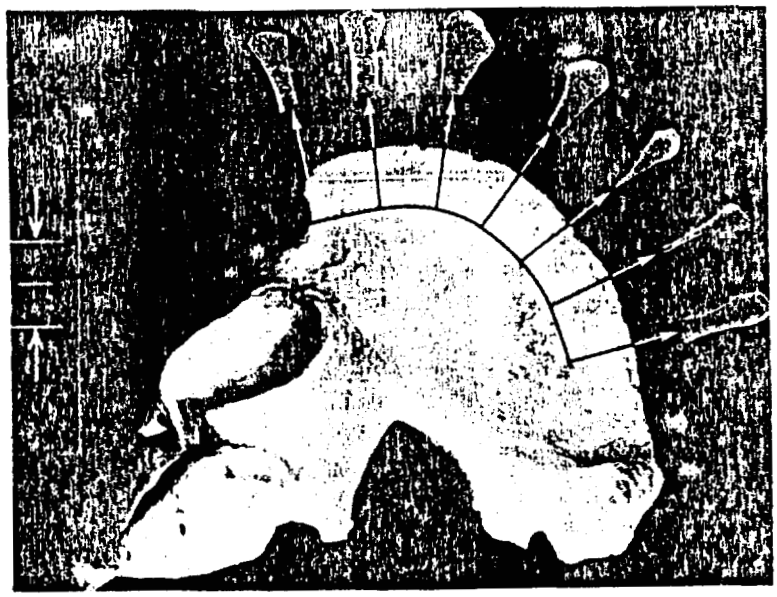

FIG. 8. Picture of the iliac crest showing the appearance of microradiographs made from thin sections of bone from different locations.

3. Calculation of Radiation Doses from Bone-seeking Isotopes. In radiation protection work the important tissues for the consideration of radiation damage from bone-seeking isotopes are thought to be in the red bone marrow found in trabecular cavities and the osteoprogenitor cells which lie close to bone surfaces. Previous calculations have been made of the doses from trabecular bone containing $^{90}Sr + ^{90}Y$ by approximating different bone shapes and sizes to spheres and slabs.[20] More recently, however, Spiers[21] has evolved a much more rigorous method for calculating the radiation dose for β emitters by assuming that β particles travel in straight lines. By calculating the energy losses of the ionizing particles as they cross the linear path lengths through bone and marrow, the dose to the bone surface cells and the marrow can be obtained. The lengths of line segments over bone and marrow given here should be directly applicable to these calculations and allow better estimates to be made of doses to a large number of different sites for all the important radionuclides which deposit in bone.

4. Estimation of Total Bone Surface in Cortical and Trabecular Bone. If we assume that the weight of the average, wet, fat-free skeleton is 4.5 kg and the density of the wet bone mineral is 2 g/cm³, we can calculate the amount of bone surface in cortical bone and in trabecular bone on the basis that 80 per cent of the skeleton is cortical and 20 per cent is trabecular.

The surface area of cortical bone

$$= \frac{80}{100} \times \frac{30\ \text{cm}^2}{\text{cm}^3} \times \frac{4500\ \text{g}}{2\ \text{g/cm}^3} = 54{,}000\ \text{cm}^3$$

or 5.4 square meters.

Correspondingly, the surface area of trabecular bone is

$$\frac{20}{100} \times \frac{130\ \text{cm}^2}{\text{cm}^3} \times \frac{4500\ \text{g}}{2\ \text{g/cm}^3}$$

$$= 5.8 \text{ square meters.}$$

This leads to a value of 11.2 square meters for the total bone surface, about half in trabecular bone and the other half in cortical bone. An average value for surface/volume ratio for the whole skeleton combining the figures for cortical and trabecular bone is

$$\frac{80}{100} \times \frac{30\ \text{cm}^2}{\text{cm}^3} + \frac{20}{100} \times \frac{130\ \text{cm}^2}{\text{cm}^3}$$

$$= 50\ \text{cm}^2/\text{cm}^3.$$

This figure allows for a calculation of total

Picture of
t showing
nce of mi-
ns made
ections of
ifferent lo-

ity of the
can calcu-
in cortical
the basis
is cortical
e

1,000 cm³

of trabec-

1500 g
g/cm³

are meters
nt half in
in cortical
ce/volume
bining the
bone is
130 cm²
cm³

n of total

TABLE 12. Perimeter, Area, Perimeter/Area, and Surface/Volume Ratios for the Iliac Crest

Section Number	Perimeter (cm)	Area (cm²)	Perimeter/Area (cm/cm²)	Surface/Volume (cm²/cm³) Pure Trabecular	% Volume	Surface/Volume (cm²/cm³) Whole Section
3	24.1	0.183	131	167	27.8	121
6	36.0	0.244	148	188	24.6	113
8	26.1	0.204	128	162	27.5	98
11	37.6	0.258	146	186	22.9	113
16	22.0	0.202	109	139	30.4	115
17	14.4	0.088	163	207	18.8	135
23	21.0	0.125	169	215	22.5	136
25	10.2	0.057	180	229	23.3	120
30	11.2	0.076	148	188	32.3	113
32	11.3	0.069	164	209	26.8	115
37	14.0	0.063	223	284	20.7	146
38	31.8	0.162	197	251	18.6	155
			Mean	183	24.6	123

Note: (Surface/Volume) is calculated by multiplying (Perimeter/Area) by 1.273—see text.

1169829

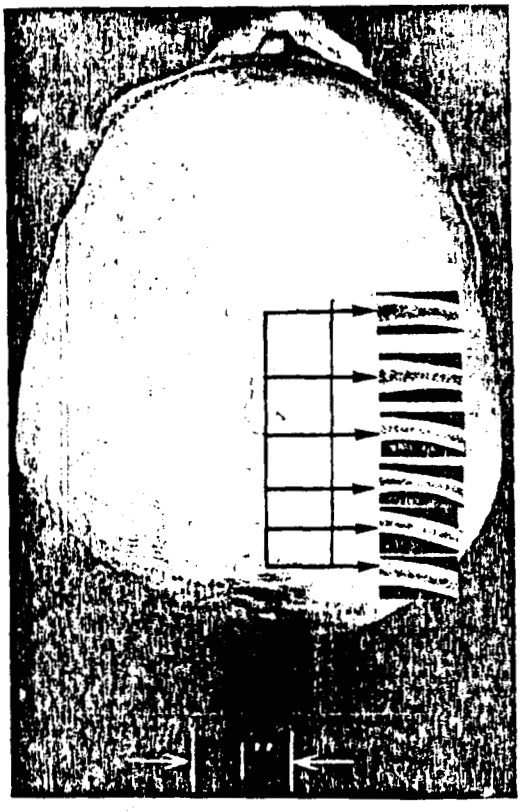

FIG. 9. Picture of the human cranium showing the appearance of microradiographs made from thin sections of bone from different sites.

bone surfaces to be made if the skeletal, fat-free weight can be estimated from body weight.

5. Extrapolation of the Relative Biological Effectiveness of Surface and Volume Bone Seekers in Animals to that Expected in Man. Our best estimations of maximum permissible doses of bone-seeking isotopes are based on a comparison with radium toxicity in man. For this reason, many experiments have been designed to relate the toxicity of different radioisotopes in experimental animals to radium. If, however, we compare the relative toxicity of a surface seeker such as ^{239}Pu to the volume seeker ^{226}Ra in the beagle dog in the absence of other modifying factors, one would only expect the relationship to be the same if the surface/volume ratio in the two species were the same.

The total skeletal surface/volume ratio for the beagle dog calculated in the same way as shown above, using 40 cm^2/cm^3 for cortical bone and 225 cm^2/cm^3 for trabecular bone (Table 15) is

$$= \frac{80}{100} \times \frac{40\ cm^2}{cm^3} \times \frac{20}{100} \times \frac{225\ cm^2}{cm^3}$$

$$= 77\ cm^2/cm^3.$$

This value is about 50 per cent higher than the value calculated for man. However, it must be regarded as only a preliminary value awaiting more extensive sampling on the beagles. It does, however, suggest that the ratio for the relative toxicity $^{239}Pu/^{226}Ra$ in the dog is lower than that which we would expect in man. In turn, the relative effectiveness of the two isotopes compared in mice would be expected to be even lower than in either dog or man. However, it is recognized that the percentage of bone surface taking part in bone formation and the extent to which this surface becomes buried may turn out to be of much greater importance in the comparison of the relative toxicity of these elements in dog and man. It is certainly apparent that we cannot take the relative toxicity in one species and apply this directly to man without consideration of all these factors together.

6. Exchangeable Bone Calcium. Rowland[18] has used quantitative autoradiography to determine the amount of calcium which is rapidly exchanged on bone surfaces when ^{45}Ca is injected into dogs and rabbits. He estimated that bone surfaces would be required to exchange an amount of calcium equivalent to that contained within 2000A of the bone surface to account for the rapid deposition of ^{45}Ca on these surfaces. If the thickness of this layer is the same in man as in dogs and rabbits, the total amount of bone calcium involved in this short-term exchange compartment in man can be calculated using the surface/volume ratio.[16] Assuming our

1169830

TABLE 13. Perimeter, Area and Surface/Volume Ratios of Bone from the Cranium of a Human Adult

Number	Perimeter (cm)	Area (cm^2)	Perimeter/Area (cm/cm^2)	Surface/Volume (cm^2/cm^3)
B 111	36.3	1.04	34	38
B 112	29.5	0.88	33	37
B 113	25.7	0.91	28	32
B 114	34.1	0.97	35	40
B 115	35.6	0.93	38	43
B 116	35.0	0.93	37	42
B 117	33.2	0.92	36	41
B 123	43.0	1.07	40	45
B 124	49.9	1.04	47	53
			Mean	41

Note: (Surface/Volume) is calculated by multiplying (Perimeter/Area) by 1.13—see text.

value of 50 cm^2/cm^3, this is 0.1 per cent of bone calcium:

$$2000 \times 10^{-6}\ \text{cm} \times 50\frac{\text{cm}^2}{\text{cm}^3} \times 100 = 10^{-1}\%.$$

This value is one-fifth of the value obtained from kinetic studies for the total short-term exchangeable calcium in man. The rest of the short-term exchangeable calcium is probably contained in soft tissues and body fluids. The importance of this short-term exchange compartment in the measurements and models of skeletal metabolism is discussed fully by Marshall.[16]

SUMMARY

An automatic scanning device has made possible for the first time the rapid quantitation of a large number of structural parameters for bone from a large number of bone sites in the skeleton. Measurements are tabulated for bones from man, dog, and mouse. Sites include femur, vertebra, ribs, iliac crest, and cranium. The parameters measured include bone volume, surface/volume ratios, linear path lengths over bone, and linear path lengths over trabecular spaces.

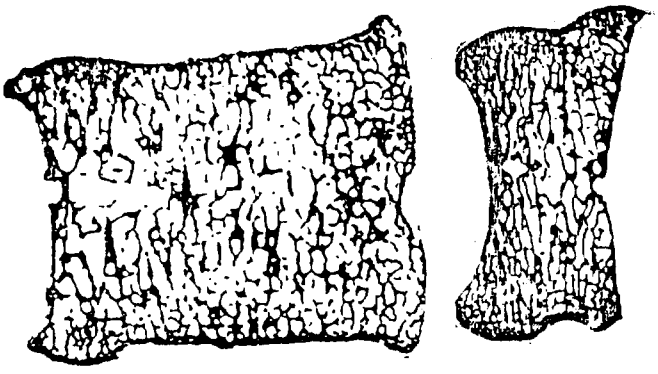

FIG. 10. Comparison of microradiographs of thin sections of bone from the vertebrae of the human, dog, and mouse skeleton.

1169831

TABLE 14. Surface Volume Ratios and Percentage Bone Volume in the Lumbar Vertebrae of 3 Dogs

Dog No.	Section	Whole Bone Section: Surface/Volume (cm^2/cm^3)	Whole Bone Section: Bone % Volume	Trabecular Bone Excluding Cortex: Surface/Volume (cm^2/cm^3)	Trabecular Bone Excluding Cortex: Bone % Volume
56	A	125	52	181	43
	B	124	55	180	43
58	A	152	38	248	30
	B	180	36	268	25
71	A	174	58	245	35
	B	120	54	228	32
	Mean	146	49	225	35

Note: (Surface/Volume) is calculated by multiplying (Perimeter/Area) by 1.273—see text.

TABLE 15. Average Line Segment Lengths Over Bone and Marrow Spaces in the Lumbar Vertebrae of 3 Dogs

Dog Number	Section	Bone: Average Line Segment Length (μ) Horizontal	Bone: Average Line Segment Length (μ) Vertical	Marrow: Average Line Segment Length (μ) Horizontal	Marrow: Average Line Segment Length (μ) Vertical
56	A	320 (212)*	420 (300)	240	330
	B	325 (218)	430 (311)	240	330
58	A	270 (162)	350 (218)	320	440
	B	220 (139)	320 (226)	380	610
71	A	230 (165)	310 (220)	250	340
	B	330 (171)	460 (254)	310	450
	Mean	282 (177)	382 (255)	290	417

* Figures in parentheses refer to the bone excluding the cortex.

1169832

TABLE 16. Comparison of Different Parameters Measured in Different Trabecular Bones of the Same Individual

	Iliac Crest	*Thoracic Vertebra*	*Lumbar Vertebra*	*Head of Femur*
Per cent bone volume	25	29	27	30
Surface/volume ratio of bone mineral	180	120	120	100
Line segment length	220 (H)*	300 (H)†	300 (H)	300
over bone (μ)	290 (V)	400 (V)	380 (V)	390
Mean	255	350	340	345
Line segment length	650 (H)	700 (H)	750 (H)	700
over marrow cavities (μ)	880 (V)	950 (V)	1010 (V)	950
Mean	765	825	880	820

* H refers to horizontal scan.
† V refers to vertical scan.

TABLE 17. Summary of Published Data on Normal Human Lumbar Vertebrae

	% Bone Volume (no. of cases in parens.)	*Sample Thickness*	*Perimeter/Area (cm/cm²)*	*Surface Area/Volume Bone Mineral (cm²/cm³)*	*Surface Area/Volume Bone Tissue (cm²/cm³)*
Dyson, *et al.*[9]	13.5 (1)	Surface only	165	207	28
Dunhill, *et al.*[6]	25 (30)	?	—	—	—
Amstutz and Sissons[10]	25 (av. 4 areas in 1 vert.)	27 μ	—	147	33
Atkinson[7]	69 (calc.) (28)	2.5 mm	—	—	—
Bromley, *et al.*[8]	15 (7)	8 μ	100	127	19
Lloyd (present study)	27 (1)	100 μ	94	120	32

1169833

These measurements are shown to have applications in the assessment of quantitative structural differences between bones, bone turnover rates, calcium kinetic studies, radiation doses from bone-seeking isotopes, and the extrapolation of the relative biological effectiveness of surface and volume bone seekers in animals to that in man. The data have been tabulated in full so that they are available to others to make their own calculations.

ACKNOWLEDGMENT

We are grateful to Dr. R. E. Rowland and Dr. J. H. Marshall who read the script and made useful suggestions. We also wish to acknowledge Joseph Farnham, Joanne Sha, and Bernice Cobb who provided excellent technical assistance.

REFERENCES

1. Amstutz, H. C. and Sissons, H. A.: The structure of the vertebral spongiosa, J. Bone Joint Surg. 51B:540, 1969.
2. Atkinson, P. J.: Variation in trabecular structure of vertebrae with age, Calcif. Tissue Res. 1:24, 1967.
3. Bromley, R. G., Dockum, N. L., Arnold, J. S., and Jee, W. S. S.: Quantitative histological study of human lumbar vertebrae, J. Gerontology 21:537, 1966.
4. Dunnil, M. S., Anderson, J. A., and Whitehead, R.: Quantitative histological studies on age changes in bone, J. Path. Bact. 94:275, 1967.
5. Dyson, E. D., Jackson, C. K., and Whitehouse, W. J.: Scanning electron microscope studies on human trabecular bone, Nature 225:957, 1970.
6. Evans, R. D.: Radium and mesothorium poisoning and dosimetry and instrumentation technics in applied radioactivity, MIT Annual Progress Report MIT-952-2, 1965.
7. Frost, H. M.: Lamellar osteoid mineralized per day in man, Henry Ford Hosp. Med. Bull. 8:267, 1960.
8. Hennig, A.: A critical survey of volume and surface measurement in microscopy, Zeiss Werkzeitschrift 30:78-87, 1967.
9. Hodges, D.: CHLOE—An Automatic Film Scanning Equipment, Hardware Reference Manual. Argonne National Laboratory Applied Mathematics Division Technical Memo Number 61, 1963. Unpublished.
10. ———: The Liberator. Argonne National Laboratory Applied Mathematics Division Technical Memo Number 127, 1966. Unpublished.
11. Jowsey, J., Kelly, P. J., Riggs, B. L., Bianco, A. J., Jr., Scholz, D. A., and Gershon-Cohen, J.: Quantitative microradiographic studies of normal and osteoporotic bone, J. Bone Joint Surg. 47A: 785, 1965.
12. Kshirsager, S. G., Lloyd, E., and Vaughan, J.: Discrimination between strontium and calcium in bone and the transfer from blood to bone in the rabbit, Brit. J. Radiol. 39:131, 1966.
13. Lee, W. R.: Bone formation in Paget's disease: A quantitative microscopic study using tetracycline markers, J. Bone Joint Surg. 49B:146, 1967.
14. Lloyd, E.: Non-Uniformity in the Retention of the Alkaline Earths in Animals and Man. Argonne National Laboratory Radiological Physics Division Annual Report, July 1968 through June 1969. ANL-7615, p. 49.
15. ———, Rowland, R. E., Hodges, D., and Marshall, J. H.: Surface to volume ratios of bone determined by computer analysis of microradiographs, Nature 218:365, 1968.
16. Marshall, J. H.: Measurements and models of skeletal metabolism. *In* C. Comar and F. Bronner (eds.): Mineral Metabolism. New York Academic Press, 1969; p. 2.
17. Rowland, R. E.: Resorption and bone physiology. *In* H. Frost (ed.): Bone Biodynamics. Boston, Little, 1964; p. 335.
18. ———: Exchangeable bone calcium, Clin. Orthop. 49:233, 1966.
19. Sissons, H. A., Holley, K. J., and Heighway, J.: Normal Bone Structure in Relation to Osteomalacia. L'Osteomalacie. Symposium published by D. J. Hioco, Masson et Cie, (eds.), 1967; p. 19.
20. Spiers, F. W.: Dose to bone from Strontium-90: Implications from the setting of the maximum permissible body burden, Radiat. Res. 28:624, 1966.
21. ———: Dose to trabecular bone from internal beta emitters. *In* Proceedings of the First International Congress of Radia-

1169834

tion Protection. Rome, Pergamon Press, 1966; p. 165.

22. Trotter, M. and Peterson, R.: Library of Congress Document ADI-7153, 1962.

23. Wakamatsu, E. and Sissons, H. A.: The cancellous bone of the iliac crest, Calcif. Tissue Res. 4:147, 1969.

APPENDIX

Different formulas have been used for the estimation of surface/volume ratios. The most common is that derived by Hennig[8]:

$$\text{surface/volume} = \frac{2n}{L}.$$

In this formula it is assumed that a body of volume, V, is intersected by a total length, L, of straight lines arranged at random in space. The total number of intersections with the specimen is n. In practice, measurements are normally made of a section in a two-dimensional plane. For these measurements a single line of length, 1, is thrown at random over the two-dimensional specimen a large number of times, and the average intercept of the length is calculated from adding up the total lengths of the intercepts and dividing by the number of intersections. The formula for surface/volume then becomes

$$S/V = 2/1_m$$

where 1_m is the mean linear intercept. This is an exact equation. However, in the system used here it has to be remembered that the length of the two-dimensional perimeter multiplied by the thickness of the section underestimates the surface area by the amount sin θ, where θ is the angle of the surface to the section plane. In the case of Haversian canals where the surfaces of the canals are at right angles to the cutting plane, sin θ = 1; hence, no correction is necessary to extrapolate from the two-dimensional to the three-dimensional surface by using the section thickness. However, in the case of trabecular bone where the trabeculae are to a first approximation orientated randomly, the average value for all angles of sin θ is $4/\pi = 1.273$. Measurement of the volume of bone, unlike the surface problem, is independent of the direction of sectioning and is always obtained by multiplying the area of the section by its thickness. Hence, for random shapes the three-dimensional parameter surface/volume is numerically equal to $4/\pi$ times perimeter/area. This relationship can be easily seen by comparing the perimeter/area of a circle with the surface/volume of a sphere.

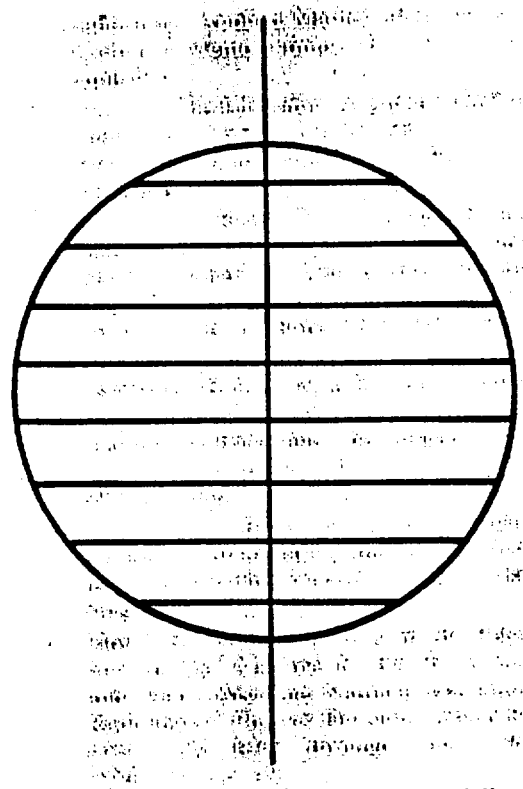

FIG. 11. Circle showing diameter and line segments.

CIRCLE

Perimeter: For the circle, the perimeter $P = \pi D$ where D is the diameter (Fig. 11). The diameter $D = n_1 d$, where n_1 = the number of line segments crossing it and d = the distance between successive lines. Each line segment gives rise to 2 intersections with the surface; therefore, $n = 2n_1$, where n = the number of intersections.

$$D \text{ therefore, } = \frac{nd}{2} \quad . \qquad (1)$$

Substituting for D in $P = \pi D$

$$\therefore P = \pi nd/2.$$

Area: The area of a circle is given by the sum of all the line segment lengths times the distance between them; i.e., area, A, = Ld, where L = the sum of all the line segments

1169835

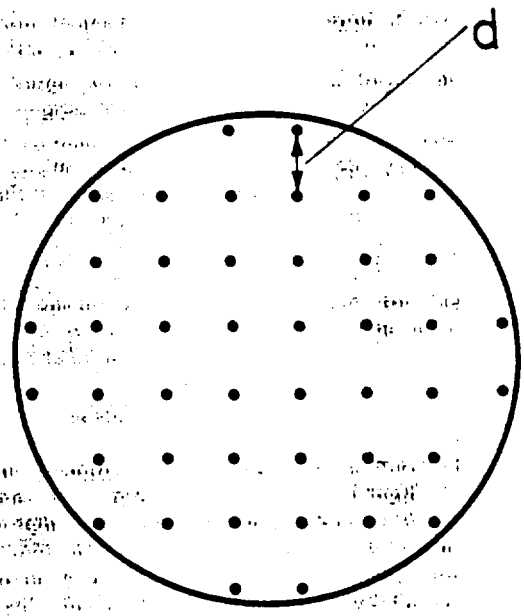

FIG. 12. Cross-section of a sphere indicating the corners of square rods seen in the cross-section.

$$\therefore P/A = \frac{\pi nd}{2Ld} = \frac{\pi n}{2L}$$

SPHERE

Surface: The surface of a sphere, $S, = 4\pi r^2$, where r = radius. Consider the volume of a sphere as being intersected by a series of rods with square cross-sections having side, d (Fig. 12), then the number of square rods through a plane of the sphere will be

$$n = \frac{2\pi r^2}{d^2}$$

$$\therefore r^2 = \frac{nd^2}{2\pi}.$$

Substituting for r^2 in the equation for the sphere surface,

$$S = 4\pi r^2;$$

this becomes

$$S = \frac{4\pi nd^2}{2\pi}.$$

Volume: The volume of the sphere, $V, = \Sigma ld^2$, where Σl is the sum of all the lengths of the square rods of side, d.

$$\therefore \text{Surface/Volume} = \frac{4\pi nd^2}{2\pi \cdot \Sigma ld^2} \text{ or } \frac{n}{2L}$$

where $L = \Sigma l$.

Comparing this formula for S/V with P/A, we see that S/V = $4/\pi$ times P/A.

1169836