# PHOTON ABSORPTIOMETRY: USE IN EXPERIMENTAL BONE TURNOVER STUDY

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Metabolic bone diseases are customarily described in terms of bone turnover (bone formation and resorption) and bone mass. Much progress in evaluating bone turnover and bone mass has been made in recent years. A number of different techniques are now available, the majority of them using the roentgenographic appearance of vertebrae or appendicular bone (*1,2*). For detailed review, the reader is referred to three symposia on the development of these methods (*3–5*). One technique in particular, the Cameron-Sorenson technique (*6,7*) of estimating bone mineral content, is attractive because it is technically simple, is nontraumatic and inexpensive, and, in addition, is free from problems of uniformity of emulsion and development which complicate many radiologic methods. The Cameron-Sorenson method has been tested in appendicular bones of a large human population and also in longitudinal studies in single individuals with conditions known to influence bone mineral content (*8,9*). In a comparison between this method and an analysis of bone in cadaver specimens, the accuracy of the method was within 3%, and in other longitudinal studies the coefficient of variation over several months was also 3% (*10,11*).

We were interested in exploring the relationship between changes in bone mineral mass measured by the Cameron-Sorenson method and changes obtained by other objective methods involving bone biopsy. The results indicated that there is a reasonably good relationship between the values measured by the Cameron-Sorenson method in the tibia and those estimated from iliac crest and ulnar biopsy specimens. Therefore, photon absorptiometry may be recommended as an adjunct in longitudinal metabolic studies of bone.

## MATERIALS AND METHODS

Ten adult female mongrel dogs, weighing approximately 13 kg each, were used; skeletal maturity in the dogs was ensured from roentgenologic evidence of closed upper tibial epiphyses. These dogs were part of another study to evaluate the effect of an increased phosphate intake on bone. The dogs were maintained on a control commercial diet* for approximately 18 months before the experimental high-phosphate diet was started. The high-phosphate intake was achieved by adding 1.6–2.0 gm of phosphate, as "Hyper-Phos-K"†, to the commercial diet. This was maintained for 40 weeks (*12*).

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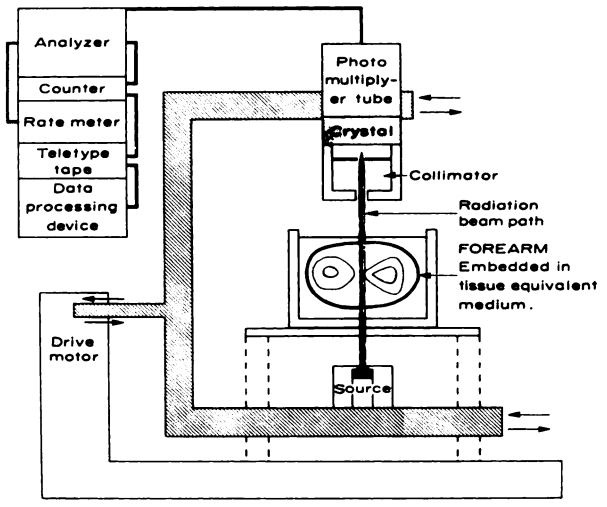


**FIG. 1.** Principle of Cameron-Sorenson bone mass determination. Photon beam is moved across limb at 4.5 cm/min; count is printed every 1.5 sec. Data can be processed by planimetry or computed electronically. Amount of bone mineral in path of beam is related to transmission counting rates. (From Riggs BL, Jowsey J, Kelly PJ, Wahner HW: Special procedures for assessing metabolic bone disease. *M Clin North Am* 54: 1061–1070, 1970. By permission of WB Saunders Co.)

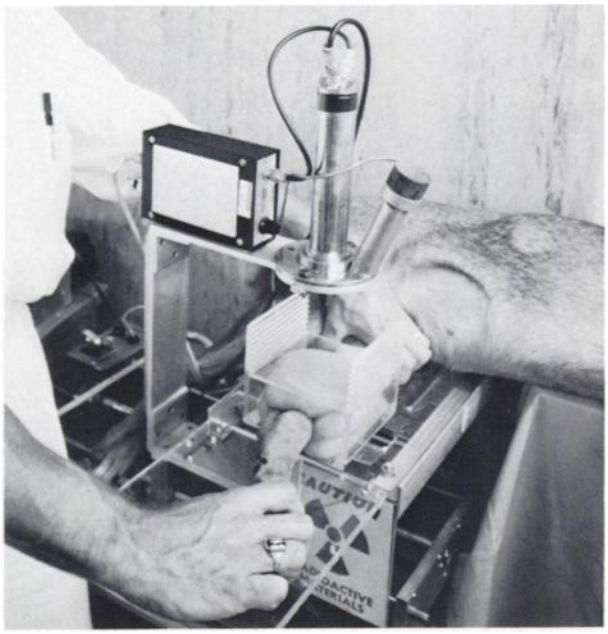

**FIG. 2.** Dog's limb in bone mineral analyzer. Adjacent to detector is light-source centering device.

Bone biopsy specimens (2 × 1 × 1 cm) were obtained from the right and left midulnar shaft and iliac crest before and at the end of the study. Surgical removal of these specimens did not affect normal ambulation. The bone specimens were dehydrated and defatted with ethyl alcohol and acetone ether. A portion of each sample was embedded in methyl methacrylate, and 100-micron-thick sections were cut with a milling machine. Microradiographs were made by using a copper target as described by Jowsey et al (*13*). Quantitative readings of bone-formation and bone-resorption surfaces were made on three nonconsecutive cross sections of each specimen of bone by a technique previously described (*13*). The mean values for bone formation and bone resorption surfaces were expressed as percentages of the total surface. Bone porosity was assessed from photographic enlargements of each microradiograph. A standard arbitrary "unit of hole" was first defined as being 20 times the size of an average osteon. This allowed us to subdivide larger holes conveniently into "units of hole" and to eliminate osteons from being counted as holes. This "unit of hole" is approximately the same for all specimens, allowing for comparison. The porous area (marrow cavity area plus hole area) was measured by planimetry and expressed as a percentage of the total cross-sectional area of bone. In addition, a sample of each ulnar biopsy specimen was ashed, weighed, and dissolved in hydrochloric acid for calcium and phosphorus determinations.

In vivo photon absorptiometry was carried out during the control period and at the end of 40 weeks of phosphate therapy. The principle of the Cameron-Sorenson method is shown in Fig. 1. The method involves the use of a monoenergetic radionuclide ($^{125}I$) as a photon source and a collimated scintillation detector pulse-height analyzer and counter. Source and detector are rigidly linked. The measured absorption is proportional to the mineral mass in the scan path after corrections for soft tissue absorption are made (*7*).

Measurements were made on anesthetized dogs (Pentobrocanal, 25 mg/kg, intravenously) laid on their left side (Fig. 2). The lateral aspect of the left tibia at the junction of the middle and distal thirds was found appropriate for measurements since here the bone is approximately circular.

In an attempt to make it possible to repeat scans in precisely the same area, the skin overlying the intended site was tattooed, and a beam of light originating from a point adjacent to the collimated crystal was used as a centering device. Furthermore, rotation of the leg had to be controlled. In the dog, the fibula joins the tibia approximately in the middle of the diaphysis and travels with it to the ankle as a flat

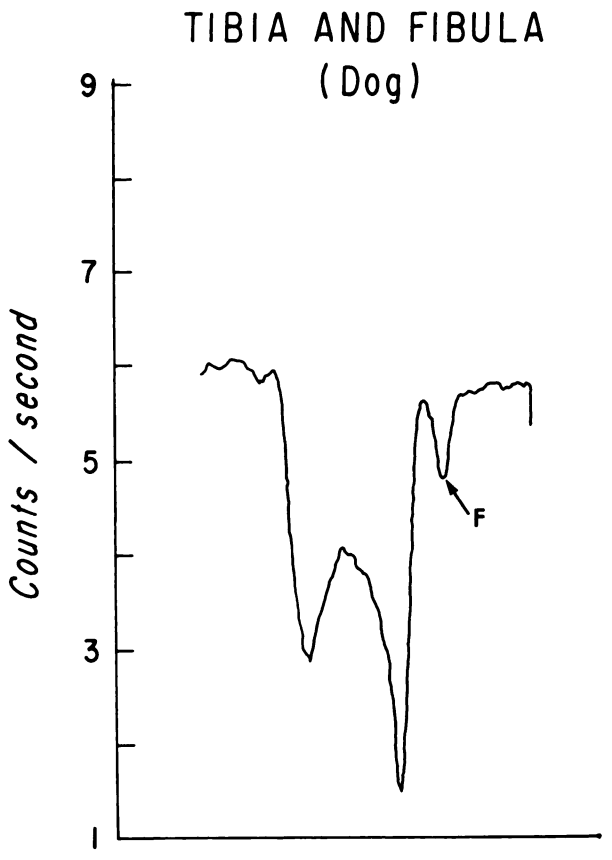


**FIG. 3.** Absorption curve showing interference by fibula which appears as small trough (F) as scan path reaches edge of tibial cortex.

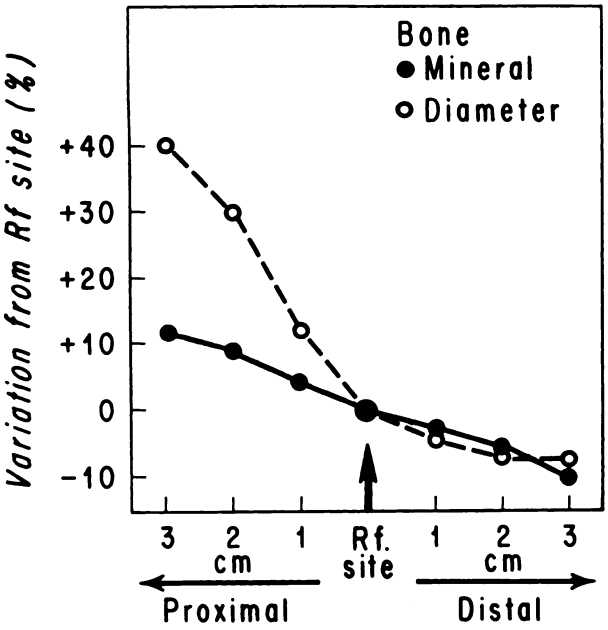


**FIG. 4.** Influence of scan location on bone mineral content and bone diameter expressed as percent change from value obtained at reference site (Rf. site) in one animal. This emphasized need of utmost care in repositioning.

structure in close juxtaposition. In a lateral projection, marked rotation brings the fibula laterally from behind, causing a second deflection on the absorption curve (Fig. 3). The x-y plot of the absorption curve was found to be of help in ascertaining consistency in placement of the tibia.

Changes in external dimensions of the bone were studied in six dogs. The precision of measurement of both bone mineral content and bone diameter was evaluated by measuring each dog three times on the same day to estimate the error inherent principally in the measuring device. This was repeated on three occasions during the control period, in which no change was anticipated, to estimate the error introduced by repositioning. The measurements were repeated after 40 weeks of the high-phosphate intake, when a change in bone mass was expected.

## RESULTS

Good reproducibility depended on the ability to repeat the measurement at the same site. Parameters such as bone mineral content and bone diameter were significantly changed with longitudinal displacement as little as 1 cm above or below the intended scanning site (Fig. 4). This occurred because both the tibia itself and the bone cortex are cone-shaped (Fig. 5). However, readings made over the same site were highly reproducible, more so without repositioning (Table 1), for both bone mineral content and bone diameter measurements.

Figures 6 and 7 show the measurements of bone mineral content and bone diameter made on three different days under control conditions and on a single day after 40 weeks of a diet supplemented with phosphates. The dogs differed widely in both mineral content and diameter. After 40 weeks of phosphate supplementation, bone mineral content decreased significantly ($p < 0.02$) but this was not the case ($p > 0.5$) for bone diameter. This statement is based

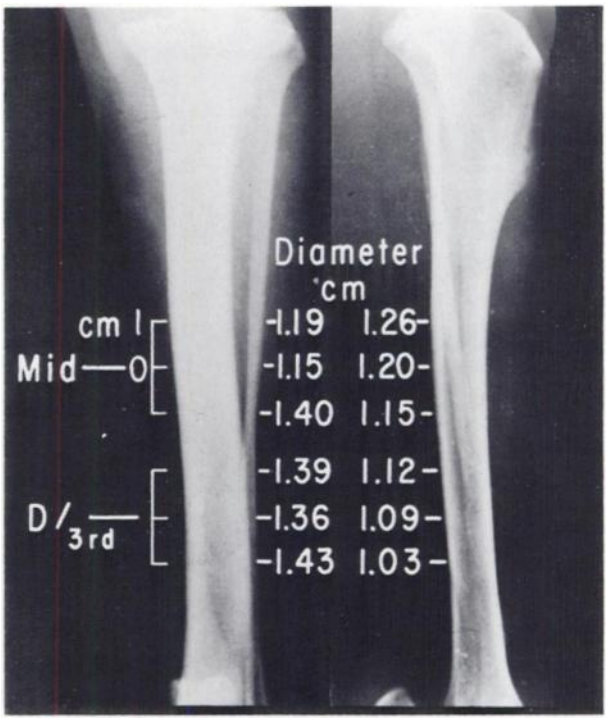


**FIG. 5.** Anterior-posterior (left) and lateral (right) roentgenograms of tibia with external diameter measurements 1 cm above or below midshaft (Mid) and distal third (D/3rd). Magnification for measurements, ×9.

**TABLE 1. VARIATION IN BONE MINERAL CONTENT AND BONE DIAMETER DURING CONTROL PERIOD**

| Measurement | Type of variation | Overall mean | s.d. | Coefficient of variation (%) |
|---|---|---|---|---|
| Bone mineral content (gm/cm) | Repeated measurements made on same day | 0.866 | 0.012 | 1.4 |
| | Repeated measurements made on three different control days | 0.882 | 0.027 | 3.1 |
| Bone diameter (cm) | Repeated measurements made on same day | 1.169 | 0.014 | 1.2 |
| | Repeated measurements made on three different control days | 1.171 | 0.028 | 2.4 |

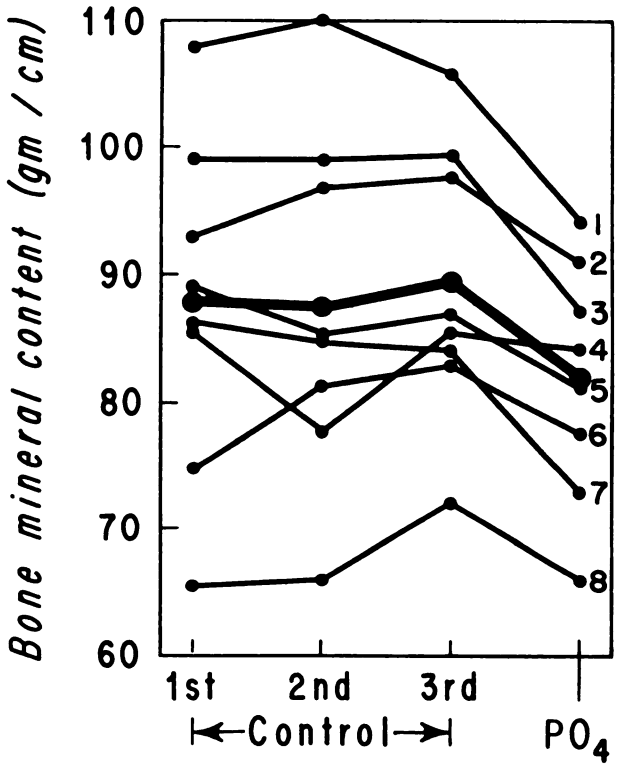


**FIG. 6.** Bone mineral mass before and 10 months after phosphate treatment. Dogs 9 and 10 were excluded from statistical analysis because measurements were made at different time intervals. Thick line represents mean value. Control data were collected over a three-week period.

on careful use of analysis of variance methods appropriate for this experiment (*14*).

Parallel to these changes in bone mineral mass, bone loss was suggested by quantitative microradiography of the biopsy samples. Bone-formation surfaces were decreased in the ulna, while bone-resorption surfaces were increased in both the ulna and iliac crest (Table 2). In addition, the cortical bone was more porous: the number of units of hole had doubled in all specimens studied and the marrow cavity-hole areas had increased by 7%. There was a positive correlation between change in bone porosity and decrease in bone mineral mass (Fig. 8). The calcium concentration per gram of ashed bone decreased 3% while the phosphorous content increased 4.8% (Table 2).

## DISCUSSION

The Cameron-Sorenson technique has proved to be a reliable and reproducible method for measuring changes in bone mass. Wentworth et al (*15*) reported the use of photon absorptiometry in growing animals, using a modified apparatus. They demonstrated this technique to be as accurate in predicting bone ash weight in animals as it is in humans. It is evident from the present studies, in which the bone mineral mass and the diameter of the bone were measured at different sites along the tibia, that exact duplication of the scan site is the most important factor in reproducibility if variation is to be kept small. Most of the variation in bone mineral and bone diameter values measured within the same day is probably the result of machine error, as reported by Wentworth et al (*15*), and is usually less than 2%. The higher coefficient of variation for long-term reproducibility is probably the result of repositioning which is more precise at short intervals than at intervals of months or years.

Microradiographic measurement of bone formation and bone resorption has proved to be a meaningful method of quantitating and defining the metabolic activity of bone. The assumption is made that a bone sample from a particular site is representative of the total skeleton in both normal dogs and those with metabolic bone diseases. Measurements in different areas of the skeleton have shown differences, but these are constant and there are predictable differences from site to site (*13,14,16,17*). Although not by itself a measure of bone mass, loss of bone is to be expected when prolonged increased bone resorption exists in the absence of a similar increase in formation. On this basis, a loss of bone mass was predictable in the dogs in the present study since bone resorption was markedly increased and formation was somewhat decreased as a result of the dietary regimen. This was reflected in the bone porosity measurements and in the bone mass.

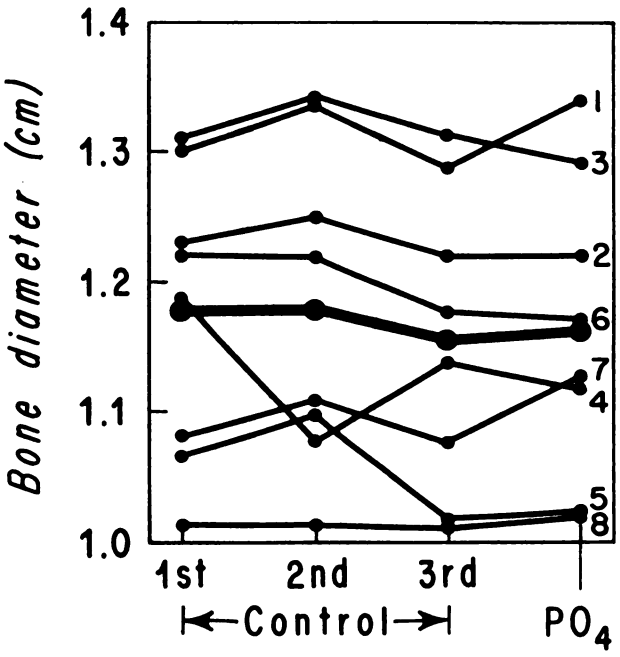


**FIG. 7.** Bone diameter before and after phosphate treatment in eight animals. Thick line represents mean value.

**TABLE 2. SUMMARY OF BONE BIOPSY FINDINGS, MEANS ± s.d.**

| Technique | Control period | After 40 weeks of phosphate treatment | p for difference from control |
|---|---|---|---|
| Mineral content | | | |
| Ulna | | | |
| Calcium (mg/gm ashed bone) | 354.2 ± 3.9 | 343.7 ± 3.3 | <0.01 |
| Phosphorus (mg/gm ashed bone) | 191.5 ± 2.3 | 200.8 ± 3.4 | <0.05 |
| Microradiography | | | |
| Ulna | | | |
| Formation (%) | 3.96 ± 0.32 | 1.55 ± 0.35 | <0.0025 |
| Resorption (%) | 3.4 ± 0.5 | 7.15 ± 1.05 | <0.0005 |
| Iliac crest | | | |
| Formation (%) | 1.8 ± 0.5 | 1.4 ± 0.3 | NS |
| Resorption (%) | 2.68 ± 0.44 | 16.7 ± 1.1 | <0.0005 |
| Ulna | | | |
| Hole units (No./section) | 70 ± 15 | 150 ± 30 | <0.0005 |
| Porous area (%) | 29 ± 12 | 36 ± 17 | <0.05 |


## SUMMARY

Photon absorptiometry is easily applicable to studies in dogs, with no major modification of an apparatus used for human studies. It is simple, atraumatic, and accurate.

In long-term studies, photon absorptiometry is a desirable adjunct to other measurements of bone mass.

Photon absorptiometry is a direct measurement of bone mass, as shown by planimetric measurement of bone components.



## ACKNOWLEDGMENT

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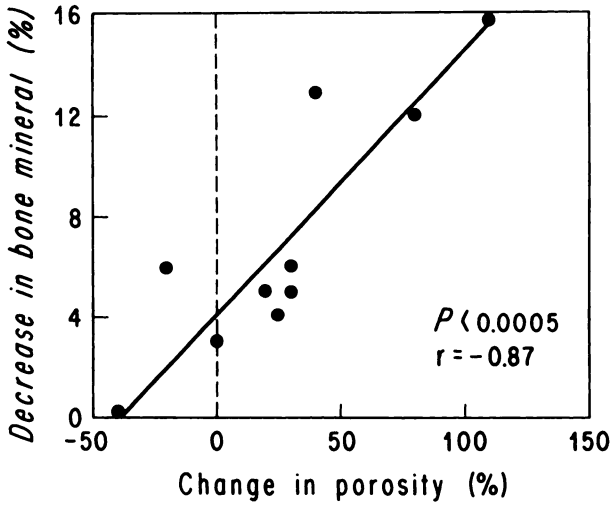


**FIG. 8.** Relationship between changes in bone mass and porosity (marrow cavity and hole areas/total sectional area).

## REFERENCES


1. MEEMA HE, MEEMA S: Cortical bone mineral density versus cortical thickness in the diagnosis of osteoporosis: a roentgenologic-densitometric study. *J Amer Geriat Soc* 17: 120–141, 1969

2. VIRTAMA P, TELKKÄ A: Cortical thickness as an estimate of mineral content of human humerus and femur. *Brit J Radiol* 35: 632–633, 1962

3. WHEDON GD, NEUMANN WF, JENKINS DW: *Progress in Development of Methods in Bone Densitometry* (NASA SP-64). Washington DC, Government Printing Office, 1965

4. WHEDON GD, CAMERON JR: *Progress in Methods of Bone Mineral Measurement.* Washington DC, Government Printing Office, 1968

5. CAMERON JR: *Proceedings of Bone Measurement Conference* (AEC Conf-700515). Oak Ridge, Tennessee, Atomic Energy Commission, 1970

6. CAMERON JR, SORENSON J: Measurement of bone mineral in vivo: an approved method. *Science* 142: 230–232, 1963

7. SORENSON JA, CAMERON JR: A reliable in vivo measurement of bone-mineral content. *J Bone Joint Surg (Amer)* 49: 481–497, 1967

8. GOLDSMITH NF, JOHNSTON JO, URY H, et al: Bone-mineral estimation in normal and osteoporotic women. *J Bone Joint Surg (Amer)* 53: 83–100, 1971

9. JOHNSTON CC JR, SMITH DM, YU PL, et al: In vivo measurement of bone mass in the radius. *Metabolism* 17: 1140–1153, 1968

10. CAMERON JR, MAZESS RB, SORENSON JA: Precision and accuracy of bone mineral determination by direct photon absorptiometry. *Invest Radiol* 3: 141–150, 1968

11. COLBERT C, MAZESS RB, SCHMIDT PB: Bone mineral determination in vitro by radiographic photodensitometry and direct photon absorptiometry. *Invest Radiol* 5: 336–340, 1970

12. LAFLAMME GH, JOWSEY J: Bone and soft tissue reaction to an increased intake of phosphate in animals. *J Clin Invest,* submitted for publication

13. JOWSEY J, KELLY PJ, RIGGS BL, et al: Quantitative microradiographic studies of normal and osteoporotic bone. *J Bone Joint Surg (Amer)* 47: 785–806, 872, 1965

*14.* BROWNLEE KA: *Statistical Theory and Methodology in Science and Engineering,* 2nd ed. New York, John Wiley, 1965

*15.* WENTWORTH RA, KALLFELZ FA, HILTZ FL, et al: In vivo estimation of bone mineral content: a research and diagnostic technique for veterinary medicine. *Amer J Vet Res* 32: 985–992, 1971

*16.* DEQUEKER J, FRANSSENS R, BORREMANS A: Relationship between peripheral and axial osteoporosis and osteoarthrosis. *Clin Radiol* 22: 74–77, 1971

*17.* NGUYEN VV, JOWSEY J: A study of bone formation in dogs of different metabolic states using autoradiographic visualization of $^{45}Ca$. *Acta Orthop Scand* 40: 708–720, 1970