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# IN VIVO ANALYSIS OF BONE CALCIUM BY LOCAL NEUTRON ACTIVATION OF THE HAND - RESULTS OSTEOPOROTIC AND HEMODIALYSED PATIENTS

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Neutron activation analysis of bone calcium in vivo, recommended by MALETSKOS in 1961 (1), was performed on man in 1962 by ANDERSON et al. (2). Developed over the last ten years in several large Centers, this technique can be used to measure the total bone mass (3,4,5) or simply the calcium mass or concentration of a bone segment, for example the hand bones (6,7,8) or vertebrae (9). For a number of reason, dosimetric (sensitivity difference between the various organs of the body towards ionising radiations), technological (difficulty of obtaining a homogeneous whole-body irradiation, high bulk and cost of the corresponding measurement equipment) but especially physiopathological we decided to use local activation technique. In generalised demineralising bone diseases, particularly osteoporosis, the calcium content variations of one bone segment are in fact comparable to those of another, and more generally to the mineral content variations of the whole skeleton (10,11,12). It is true that ideally we should measure the mineral content of the lumbar vertebrae where the metabolic activity is especially high, and where damage may occur sooner or in any case is detected earlier in osteoporosis. However neutron irradiation of the vertebrae meets with certain technical problems and may also present difficulties in the interpretation of results, especially if serious aortic calcifications are present as quite often happens in osteoporotic cases. Furthermore in other bone diseases, hyperparathyroidism for instance and especially renal osteodystrophy, bone mineral loss is particularly premature and pronounced in the hand (13,14,15) and we therefore decided for the moment to use the hand for the neutron activation analysis of bone calcium. The technique enabled us to measure the calcium concentration of the hand bones in hemodialysed subjects and in patients with primitive osteoporosis.

## I - MATERIAL AND METHODS

### - Principle

When bombarded with thermal neutrons calcium 48, a stable calcium isotope of very low natural isotopic abundance (0.185 %), is converted into radioactive calcium 49 which disintegrates with an 8.8 min half-life emitting high-energy gamma photons (3.10 MeV). The gamma radiation intensity, measured with sodium iodide scintillators, is proportional to the mass of stable calcium exposed to the neutron flux. By comparing the induced calcium 49 activities with those of a standard of known chemical composition, irradiated and measured under identical conditions, it is easy to measure the mass of stable calcium contained in the bone segment examined.

### - Irradiation

The irradiation is performed with neutrons emitted by isotopic sources of californium 252 (252 Cf), ideally suited to the measurement of calcium by local activation (16).

In the hand the mass of soft tissue standing between the neutron source and the bone is small (the ratio of bone tissue volume to total irradiated volume is 0.17 against 0.08 for the whole body). On passing through these soft tissues the slow neutrons chiefly emitted by californium ($E_n$ = 1.5 MeV) change into thermal neutrons, responsible for the (n,γ) reaction on stable calcium.

A relatively homogeneous irradiation is achieved by the use of two 200 µg 252 Cf sources arranged diametrically on a vertical ring of 12 cm diameter. The thermal flux obtained ($7 \times 10^5$ $n.cm^{-2}.s^{-1}$), measured with gold dosimeters, varies over a path of 10 cm by about 5 % on an axis perpendicular to the source plane and by less than 3 % on an axis in the source plane. This irradiation device, described in detail earlier (8) is placed at the centre of a 1-metre paraffin cube covered by a 2 mm cadmium envelope and protected by 5 cm thick lead shielding. Under these conditions the dose rate equivalent measured at the surface of the lead castle is less than 3 mrem per hour.

The subject grasps a horizontal plastic handle which slides along rails inside the irradiation block and locks into the activation position where the source plane coincides with that of the metacarpus-phalange joints (fig. 1). To limit irradiation to the hand bones alone the subject's forearm is protected by a cadmium bracelet, its lower edge coinciding with the line joining the radial and cubital styloids.

- Induced radioactivity measurement

After a 5-minute irradiation the subject gains the measurement room and grasps a handle fixed between two 5" x 5" sodium iodide NaI(Tl) scintillators 7 cm apart (fig. 2). The time between the end of irradiation and the start of counting is generally no more than 45 seconds. The radioactivity thus induced in the hand is recorded for 32 minutes between 1.10 and 3.5 MeV by means of a multichannel γ spectrometric analyser

(INTERTECHNIQUE DIDAC 4000). After background substraction and smoothing of the residual spectrum the 49 Ca radioactivity is obtained by integration of the 3.10 MeV peak impulses freed from contributions due to 24 Na and 38 Cl (about 10 % in normal subjects).

### - Standards

The standards consist of skeleton hands of known chemical composition, coated with paraffin and arranged in a functional anatomical way around a handle identical with those used for in vivo irradiation and for measurement. The calcium mass contained in each bone of these standards was determined before hand by high-resolution $\gamma$ spectrometry after pile irradiation. To study the influence of the bone volume and soft tissue thickness on the induced 49 Ca activity eight different standards were built, their bone volumes ranging from 40 to 80 $cm^3$ and the bone-soft tissue (paraffin equivalent) volume ratios from 0.17 to 0.28. The standard deviation calculated on the recorded activities per gram of calcium for these different phantoms is ± 2.7 %. This means that under the experimental conditions described, and within the limits of anatomical variations, the results may be taken as independent of the morphology of the hand examined.

### - Bone volume determination

The bone volume of the hand is measured indirectly. From preliminary measurements carried out in vitro the relationship was established between the volume of the bones and their projected surface area, which is itself determined by planimetry on an X-ray image of the hand taken in dorsopalmar incidence on KODAK No.RPR X omatic G film.

### - Choice of subjects

The study covered two groups. The first numbered 45 subjects, 28 women and 17 men, all over 40 and attacked by primitive osteoporosis observed radiologically by at least one vertebral collapse. The second group consisted of 46 azotemic patients, 20 women and 26 men under hemodialysis for at least two years. The patients were separated into three categories according to wether signs of hyperparathyroidism were present or not, especially in the hand : 1°) Cases of unequivocal hyperparathyroidism ; 2°) Cases of unobtrusive or doubtful hyperparathyroidism ; 3°) Cases in which no radiological signs of hyperparathyroidism were observed. All others radiological signs of renal osteodystrophy, in fact rarely observed in this group, were deliberatly neglected.

The calcium concentration of the hand bones of each osteoporotic or hemodialysed patient was compared with the average value obtained on the reference subjects of corresponding age and sex (17).

## II - RESULTS

The total measured bone calcium referred to the calculated bone volume corresponds to the volumic bone calcium concentration expressed in grams of calcium per cubic centimetre of bone (g Ca/cm$^3$ bone).

### - Osteoporotic subjects

As shown by figures 3 and 4 the bone calcium concentration is significantly lower than that of the control subjects in four out of six osteoporotic women under 60, in seven out of fifteen aged between 60 and 70 and in only one out of seven over 70. The same phenomenon is observed in six osteoporotic men out of ten under 60 and in five out of six aged between 60 and 70.

### - Azotemic subjects

Among the twenty women under hemodialysis (fig. 5) seven had radiological signs of severe hyperparathyroidism and in two cases the hands were badly affected : all had a very low hand calcium concentration, significantly below that of the standards ; four has doubtful signs of hyperparathyroidism, three of these had a low calcium concentration and only one had a normal calcium concentration. Nine women had no signs of hyperparathyroidism and their calcium concentration was not different of controls. Of the twenty six men under hemodialysis (fig. 6), four without radiological signs of hyperparathyroidism and four with doubtful signs nevertheless had a calcium concentration significantly lower than that of controls.

## III - DISCUSSION

### 1 - Methods

Calcium content measurements of the hand bones were obtained with a satisfactory precision, sensitivity and reproducibility by the local neutron activation technique. The statistical accuracy on our 49 Ca measurements (about 4000 counts per 32 minutes in an average subject) is ± 2 %. Over a series of ten analyses carried out on a phantom (paraffin-clad skeleton hand) the standard deviation is ± 2.4 %. The reproducibility in vivo was calculated after normalisation of results obtained from two or three successive measurements performed on ten volunteers and the variation coefficient with respect to the average does not exceed ± 2.4 %. This similarity between results recorded in vivo and in vitro shows that owing to precise repositionning during irradiation and measurement, no extra variable is introduced by neutron activation of the human hand. The only reaction liable to interfere with the 49 Ca measurement is the (n,p) reaction transmuting stable chlorine into radioactive 37 S, a 3.10 MeV $\gamma$ emitter, but in fact the irradiation of an organic chlorine solution showed that under the experimental conditions described this interference is negligible.

Moreover the method is quite harmless since the amount of radiation delivered to the subject is small, as shown by absorbed dose measurements carried out in a cylindrical plastic phantom. The total neutron and $\gamma$-ray dose measured with an ionisation chamber made of tissue-equivalent plastic is 0.28 rad/min. The gamma radiation dose estimated by the use of thermoluminescent detectors is 0.2 rad/min, the difference between the two measurements corresponding to the neutron dose alone. Assuming a quality factor 8.5 for neutron (18) the adsorbed dose equivalent on the hand due to neutrons and gamma rays is about 2.1 Rem/min. On the basis of these figures the irradiation time was set at 5 minutes, which corresponds to an absorbed dose equivalent of about 10 Rem on the hand. This dose, strictly localised, compares favourably with those commonly delivered during conventional radioisotopic examinations such as thyroid uptake tests. Moreover for purposes of comparison it should be remenbered that according to French norms the maximum dose equivalent acceptable on the hand, for individuals directly engaged in work under radiation, is 60 Rem a year or 15 Rem a quarter. The examination may therefore be repeated quite safely three or four times a year.

The choice of the hand bones for neutron activation was made for reasons of technical convenience since it may be assumed that the mineral content of the peripheral and axial bones correlate well enough to allow the mineral content of the vertebrae to be estimated from that of the hand bones. A significant relationship between peripheral and spinal bone mineralisation has in fact been observed by various methods : DOYLE (19) observes a good correlation between the weight of ash per unit volume of the third lumbar vertebra and the mineral content of the ulna.

WILSON (20) finds a significant relationship between the bone mineral content of the radius and the mineral content of the thoracic vertebrae measured by photon absorptiometry ($r = 0.60 - 0.70$). COHN et al. (10) find a highly significant correlation ($r = 0.94$) between the bone mineral content of the radius and the whole body calcium measured by neutron activation ; the same correlation ($r = 0.94$) was observed recently by MEEMA et al. (12) between the bone mineral content of the radius and the calcium mass of the trunk measured by local neutron activation.

The neutron activation measurement of bone calcium appears to give better results than the monoenergetic photon absorptiometric measurement of bone mineral content (B.M.C.). It is true that the correlation between the hand calcium content Ca and the radial epiphysis B.M.C. measured by us in 37 normal subjects by CAMERON's technique (21) is strong ($r = 0.87$ ; $p < 0.001$), the relationship being as follows : B.M.C. = 0.070 Ca + 0.262. However the photon absorptiometric measurement of the radial epiphysis B.M.C. gives less reliable results in patients suffering from demineralising bone diseases as shown by the lower correlation factors we observed in groups of osteoporotic ($r = 0.85$ ; $p < 0.001$) (figure 7) and hemodialysed patients ($r = 0.72$ ; $p < 0.001$) (figure 8). MEEMA et al. (12), COHN et al. also found a poorer correlation between the radius B.M.C. and the trunk or whole body calcium in osteoporotics. Moreover we were obliged to abandon the absorptiometric B.M.C. measurement in certain patients suffering from particularly severe bone loss, most of the results from several consecutive measurements being impossible to interpret. In addition the reproducibility of radial epiphysis B.M.C. measurements carried out on a given subject was found to be not so good as these obtained in the measurements of calcium by neutron activation : thus, the reproducibility of the absorptiometric technique, calculated after normalisation of results obtained on ten control volunteers measured once a week for four to six weeks is only

± 3.6 %. We feel therefore that while the use of monoenergetic photon absorptiometry for bone tissue analysis is usefull in epidemiological studies covering a wide population, such as those carried out for instance by SMITH et al. (22), local neutron activation is preferable in longitudinal studies on a single individual.

2 - Osteoporosis is best defined anatomically as the rarefaction and attenuation of the trabeculae of cancellous bone combined with attenuation of the cortical thickness. However our osteoporotic subjects were chosen arbitrarily on the basis of a radiological criterion : the presence of at least one vertebral collapse, which means that all cases where bone loss had not yet caused a vertebral fracture were excluded from this category.

At the same time there is no doubt that in a variable proportion of the subjects taken as controls some bone degeneration due to age was already present and this proportion is certainly higher in women of at least 60, the age at which bone loss is rapid (22,23,24,25,26), than in young women and in men who lose little bone even on ageing. It is not surprising therefore that the calcium concentration of the hand bones in a large number of our osteoporotic cases does not significantly differ from that of the control subjects in the same age group, particularly when over 60. Similar observations were made by GOLDSMITH et al. (27) measuring the bone mineral content of the radius by photon absorptiometry.

Conversely, the great majority of our osteoporotic cases under 60, eight men out of eleven, four women out of six, had significantly lower hand calcium concentration than the controls. Of course it is likely that beyond a certain degree of bone atrophy the risk of vertebral fracture increases considerably as shown by COURPRON et al. (28) or SMITH et al. (22). In favour of this hypothesis the fact that a distinctly lower proportion of men than women over 60 suffer from vertebral collapse, the degree of bone atrophy at which this occurs being the same in both sexes but the average bone loss being smaller in men than in women of comparable age and especially after 50-60. However the risk of vertebral fracture is difficult to estimate as a function of bone atrophy because other factors are involved, particularly the causal injury and the mechanical quality of the bone. BELL et al. (29) have shown for example that the load necessary to break lumbar vertebrae decreases much more quickly than their mineral content. A better criterion for the definition of osteoporosis is therefore a lower bone atrophy limit beyond which the subject runs a serious risk of vertebral fracture rather than its random occurence.

3 - For patients under hemodialysis, the volumic hand calcium concentration measurement appears more "discriminating" than X-rays to detect the bone mineral loss, as already observed by CATTO et al. (13). Like these authors we consider this method superior to photon absorptiometry as shown by our comparative measurements on 21 patients : the correlation coefficient is lower than that found in control and osteoporotic subjects while in the same way as in osteoporotics the measurements were impossible in subjects with signs of severe hyperparathyroidism at the hand.

However we do not know wether the hand bone mineral loss as evaluated by neutron activation is directly representative of bone atrophy in osteoporosis or in renal osteodystrophy. The phenomena are usually estimated by semi-quantitative histological measurements of the trabecular bone volume (T.B.V.). As shown by the measurements we made on a transiliac biopsy in 22 osteoporotics by means of BORDIER's technique (30) there is a weak correlation between the iliac T.B.V. or the iliac T.B.V. minus the trabecular osteoid volume (T.B.V. - T.O.V.) and the volumic hand calcium concentration |Ca| (figures 9 and 10).

In hemodialysed patients two facts are observed : the correlation coefficient between hand calcium concentration and absolute bone volume minus osteoid volume (r = 0.34 - figure 11) is distinctly better than accounting for absolute bone volume alone (r = 0.14) ; a fairly good correlation is obtained between the hand calcium content and an osteoclastic resorption index : the number of osteoclasts per $mm^3$ in trabeculae in cancellous bone (figure 12). This comparison, in patients under hemodialysis, between bone histology and neutron activation data suggests that the mineral loss of the hand bones is probably due to both a mineralisation defect and an osteoclastic hyperresorption due to hyperparathyroidism. On the other hand, it is possible that these low correlation coefficients found in osteoporotic and in azotemic patients are chiefly bound up with the difference in bone structure between the two measurement sites, histological measurements accounting in particular for the cancellous bone only. In any case, the neutron activation measurement of the volumic hand bone calcium concentration is in our opinion an ideal method to evaluate bone loss although it only gives an indirect estimate it has the advantage over transiliac bone biopsy of being non invasive. Furthermore it is reproducible, as shown above, whereas the reproducibility of semi-quantitative histological measurements of the T.B.V. is open to criticism (30,31,32). We ourselves have checked the reproducibility of histological T.B.V. measurements using results obtained by GIROUX (33) on transiliac bone samples taken from 42 control subjects of both sexes. The mean difference between two T.B.V. measurements carried out on two symmetrical iliac samples, left and right (GIROUX, zone III), in a given subject was 26.69 % with a variation coefficient of 29.99 % (range 1-172 %). Owing to its good reproducibility the hand bone calcium content measurement ought to give an accurate picture of the development of bone mineral loss in each individual suffering from demineralising bone diseases, and above all a precise idea of the efficiency of treatments aimed at slowing down or even eliminating these symptoms.

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# FIGURE LEGENDS

Figure 1 - Schematic view of the irradiation device showing the hand in the irradiation position. Note the cadmium bracelet used to protect the subject's forearm.

Figure 2 - Schematic view of the subject's hand during the recording of the induced radioactivity. The plastic handle is used to obtain precise repositionning during successive measurements.

Figures 3 and 4 - Volumic bone calcium concentrations |Ca| obtained from primitive osteoporotic patients, 17 men (fig. 5) and 28 women (fig. 6), of different ages. The S.D. for the normal contrast group in the same decades are indicated.

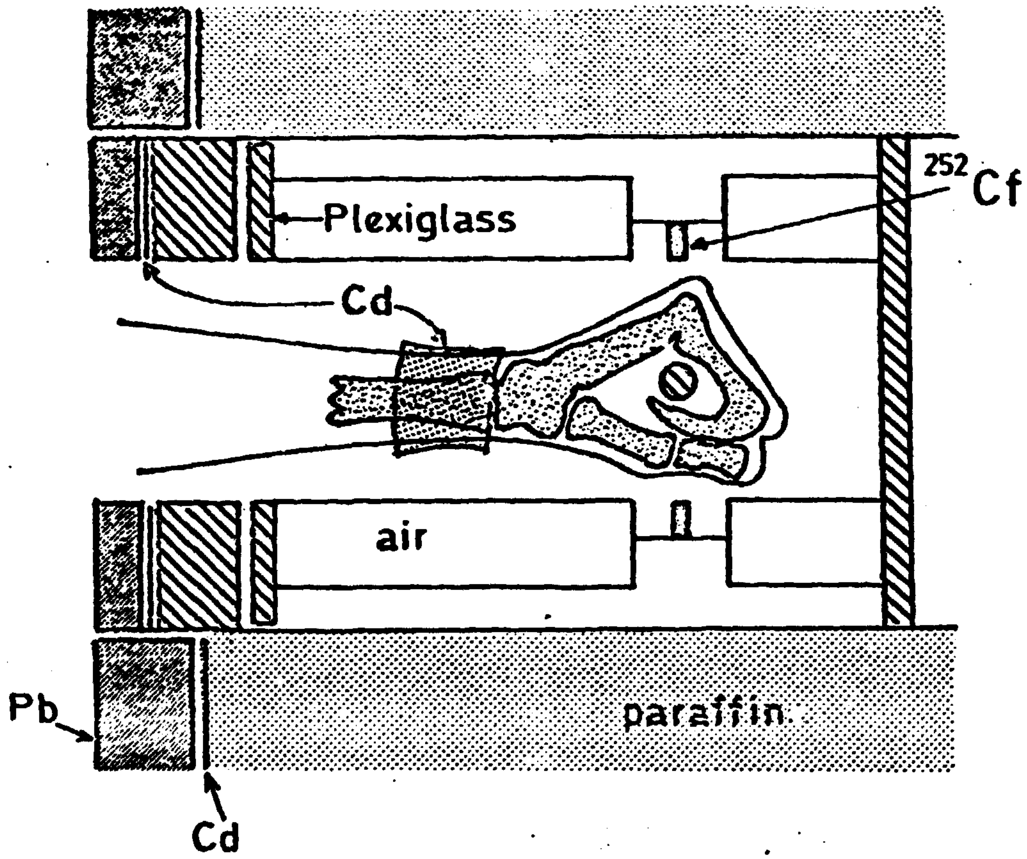
Figures 5 and 6 - Volumic bone calcium concentration |Ca| obtained from hemodialysed patients 26 men (fig. 5) and 20 women (fig. 6) of different ages. The S.D. for the normal contrast group in the same decades are indicated : Open circle = cases of unequivocal hyperparathyroidism ; asterisk = cases of unobtrusive or doubtful hyperparathyroidism ; black circle = cases with no radiological signs of hyperparathyroidism.

Figure 7 - Correlation of calcium content of the hand with radial bone mineral content (B.M.C.) in 13 osteoporotic patients. The S.D. for the normal control group is indicated.

Figure 8 - Correlation of calcium content of the hand, Ca, with radial bone mineral content (B.M.C.) in 21 hemodialysed patients. The S.D. for the normal control group is indicated.

Figures 9 and 10 - Correlation of apparent volumic calcium concentration of hand bones |Ca| with trabecular bone volume - T.B.V. - (fig. 9), or trabecular bone volume minus trabecular osteoid volume - |T.B.V. - T.O.V.| - (fig. 10) measured on a transiliac biopsy in 22 osteoporotic patients.

Figures 11 and 12 - Correlation of apparent volumic calcium concentration of hand bones |Ca| with trabecular bone volume minus trabecular osteoid volume |T.B.V. - T.O.V.| (fig. 11) or number of osteoclasts per $mm^3$ |ost| (fig. 12) in 21 hemodialysed patients.



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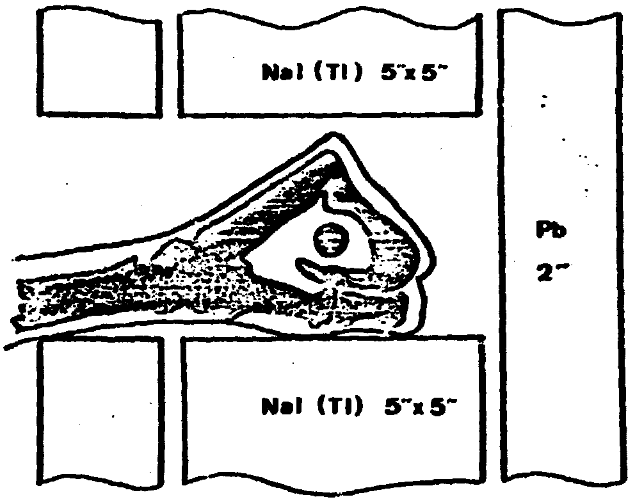

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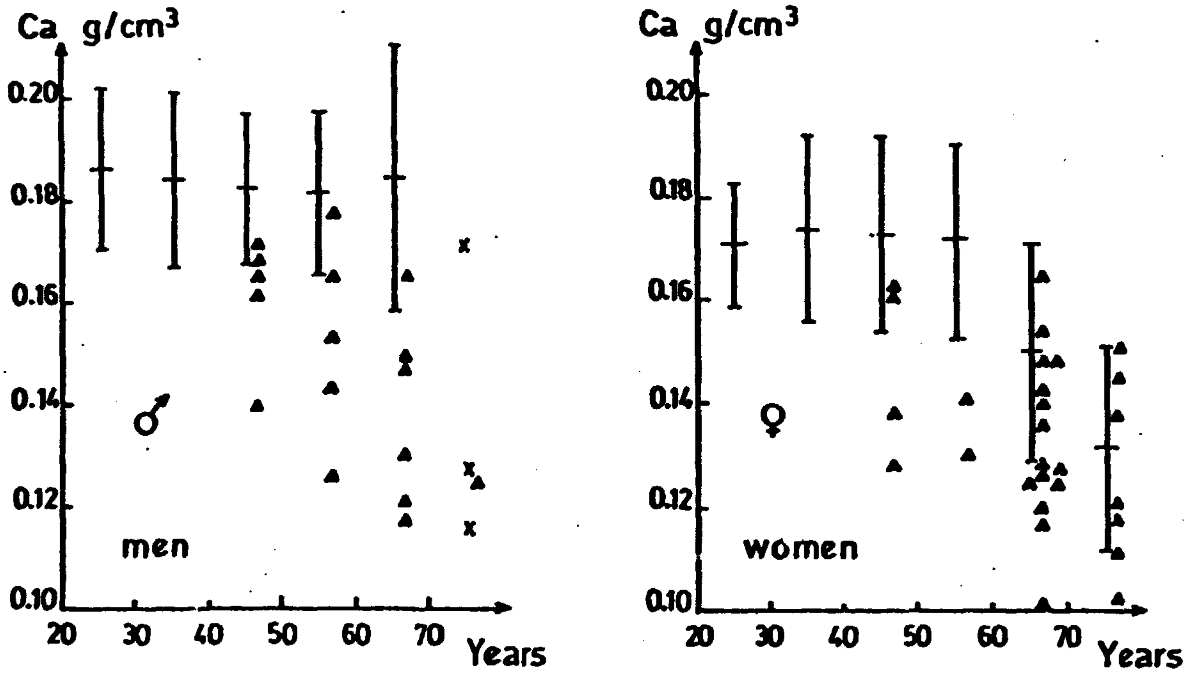

OSTEOPOROSIS

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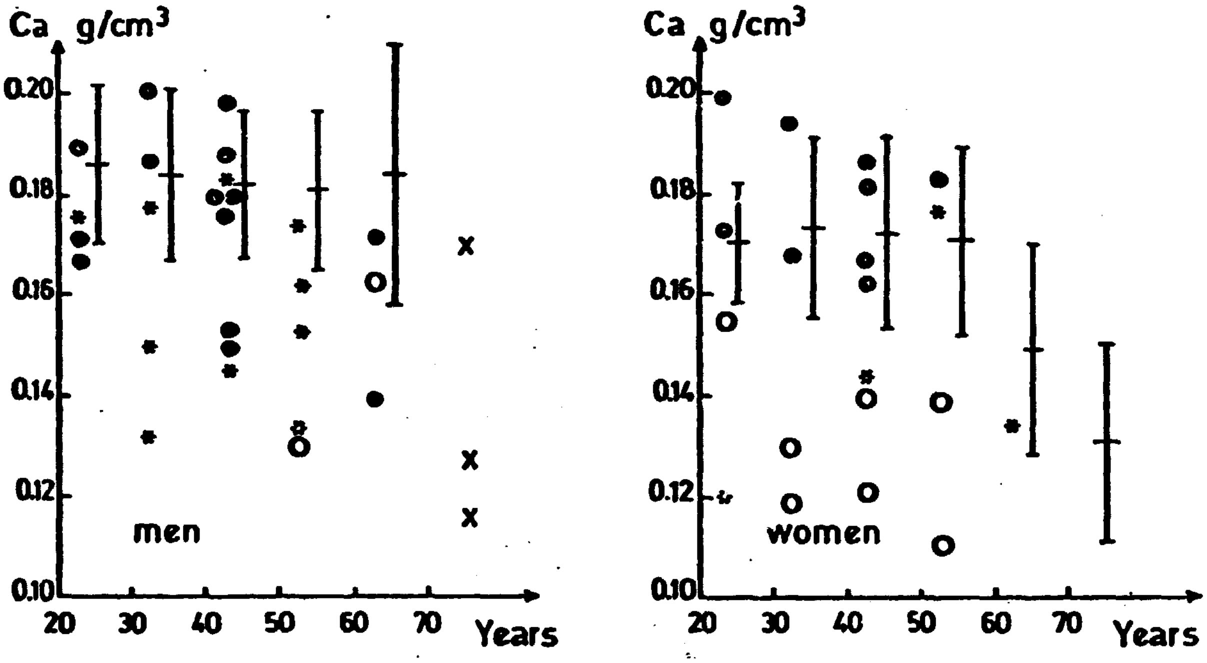

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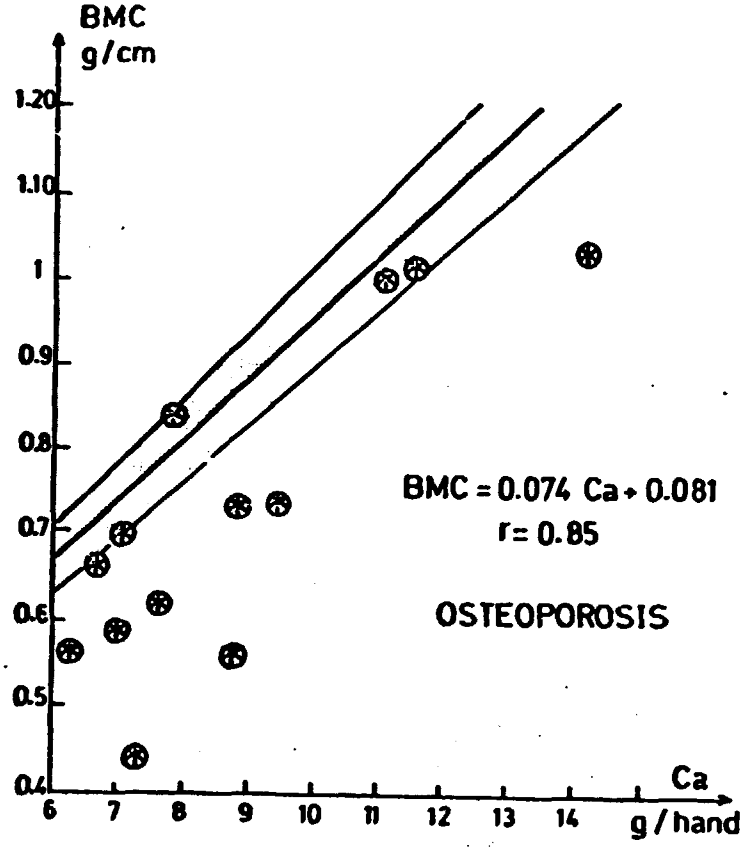
Figures 5 and 6 - Volumic bone calcium concentration |Ca| obtained from hemodialysed patients 26 men (fig. 5) and 20 women (fig. 6) of different ages. The S.D. for the normal contrast group in the same decades are indicated : Open circle = cases of unequivocal hyperparathyroidism ; asterisk = cases of unobtrusive or doubtful hyperparathyroidism ; black circle = cases with no radiological signs of hyperparathyroidism.



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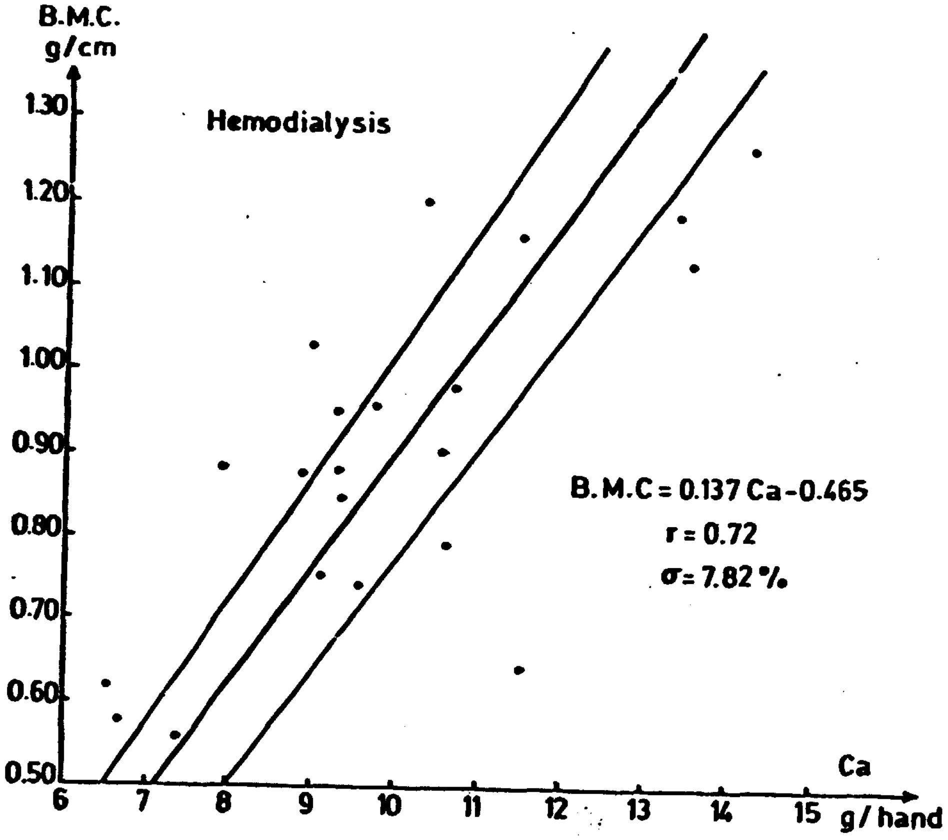

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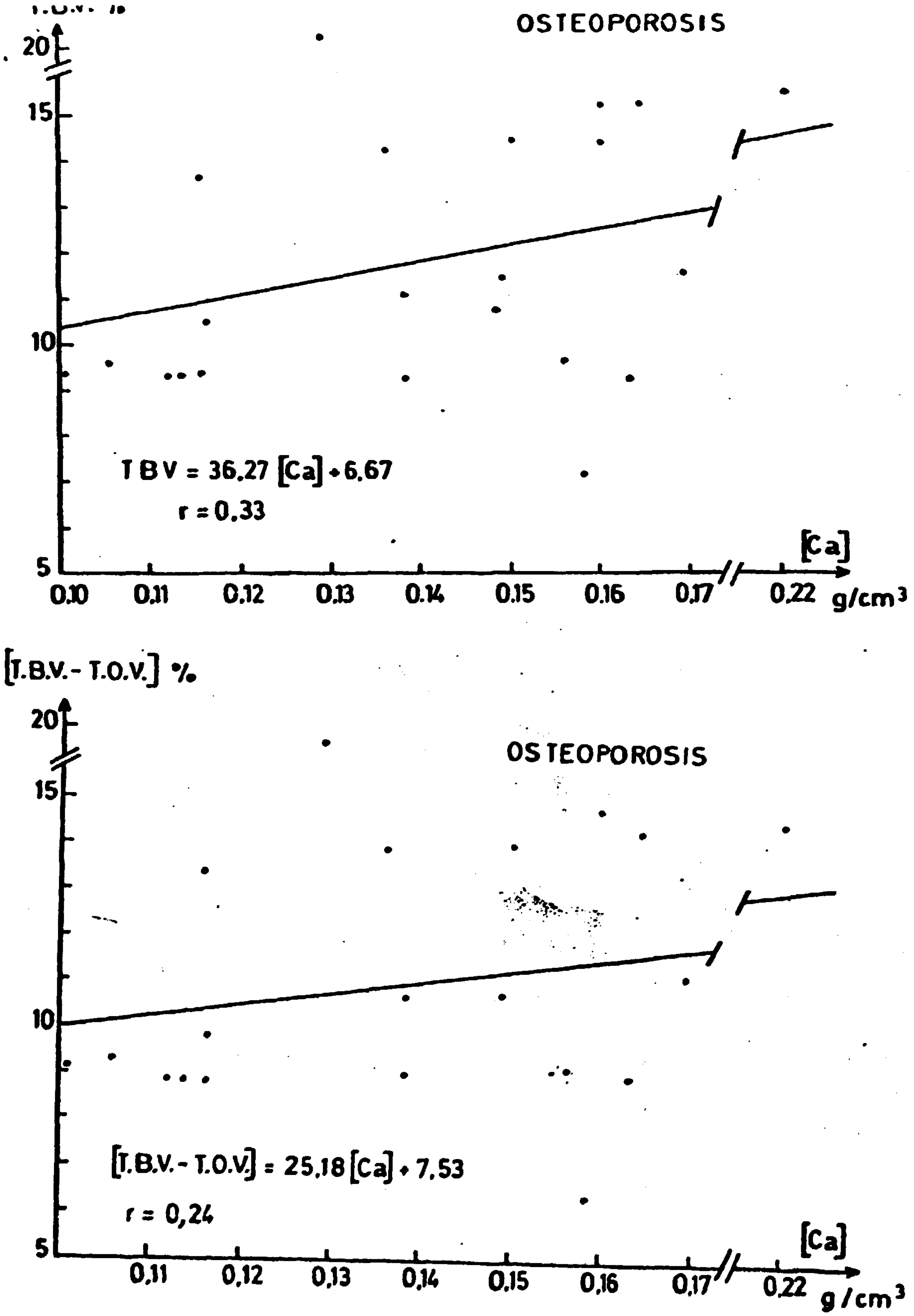

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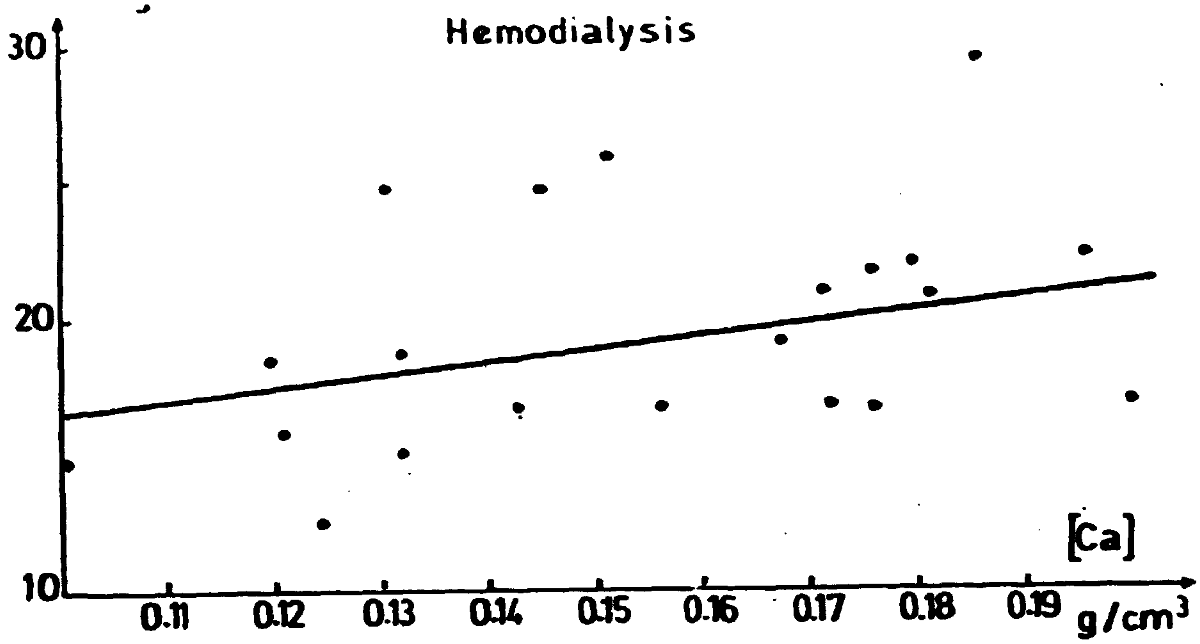
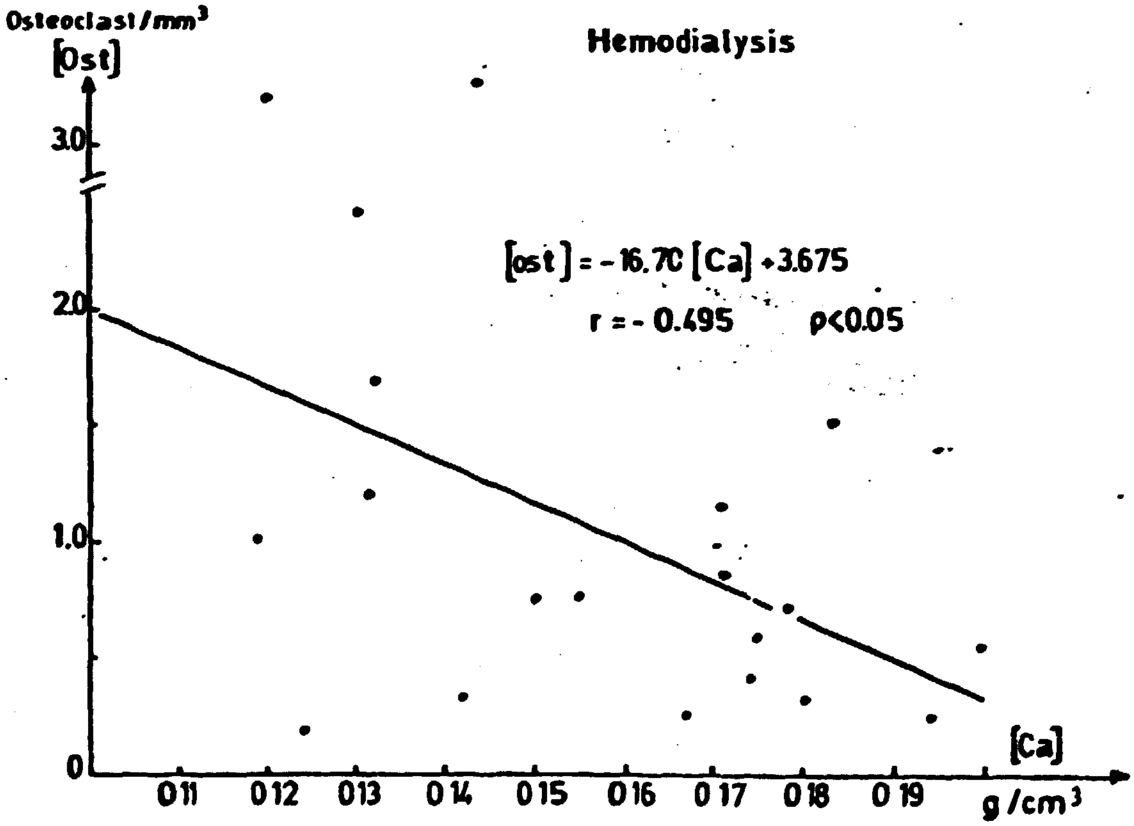


$$[T.B.V.-T.O.V.] = 45.65[Ca] + 12.13$$
$$r = 0.34$$



Figures 11 and 12 - Correlation of apparent volumic calcium concentration of hand bones |Ca| with trabecular bone volume minus trabecular osteoid volume |T.B.V. - T.O.V.| (fig. 11) or number of osteoclasts per mm³ |ost| (fig. 12) in 21 hemodialysed patients.