IN VIVO ANALYSIS AT THE SERVICE HOSPITALIER FREDERIC JOLIOT (1965 - 1981)

by

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ABSTRACT

In the first chapter, historical development of In-vivo Neutron Activation Analysis (I.V.N.A.A.) at the Service Hospitalier Frédéric Joliot is presented. Then after having reviewed the preliminary animal experiments, the author explains the choice of the irradiation facilities used for human partial I.V.N.A.A.. The clinical applications of this technique in the field of thyroid and bone metabolisms are described. The clinical results obtained in patients suffering from various demineralizing bone diseases (osteoporosis, renal osteodystrophy) are given in detail.

1. HISTORICAL DEVELOPMENT

Adventure of In-Vivo Neutron Activation Analysis (I.V.N.A.A.) at the Service Hospitalier Frederic Joliot begins in 1965 at College Station-Texas.

On the occasion of a post-meeting session following the second Conference on Modern Trends in Activation Analysis, Dr. Comar listened to Dr. Lenihan evoking the possibilities of measuring in-vivo thyroid iodine. Having a presentiment of the fine future of the in-vivo measurements described the previous year by Anderson et coll. (1), he decided to explore this new field of analysis. The first experiments dealing with the estimation of animal thyroid iodine were begun immediately and two years later, in 1967, the first results were published in Nature (2).

In the next eight years, I.V.N.A.A. was essentially directed towards bone mineral concentration measurement, first in animal then in man, and in 1975 the first clinical applications of this technique, dealing with bone calcium determination, were performed.

Nowadays, serial measurements of bone calcium concentration are routinely taken on patients suffering from various demineralizing bone diseases to determine the course of their diseases and the response to the therapeutic treatment.

2. ANIMAL EXPERIMENTS

In 1972, 14 MeV neutrons were used for animal experiments (3). To ensure an irradiation as homogeneous as possible, the anesthetized rats were placed in an animal holder rotating along two perpendicular axes at a rate of several times per minute. This apparatus, surrounded on all sides by a 10 cm thick paraffin construction, faced the neutron target of a Cockroft-Walton generator. At the end of the irradiation time (3 min) the animals were removed from their containers and the induced radioactivity (^{28}Al due to transmutation of natural phosphorus, ^{13}N, ^{38}Cl, ^{24}Na and ^{49}Ca) was measured with a 12,7 cm x12,7 cm NaI (Tl) crystal. The main value of such a technique is that the changes in the elemental composition of the organism during the various events of life - such as puberty, suckling, senescence - can be followed in time on the same animal.

In 1973, using a filtered fission neutron beam, total potassium (4)was measured in-vivo in small animals like mice in registring the 29.8 KeV capture gamma-ray. (Potassium measurement by counting ^{40}K radioactivity is not possible with small animals because the total mass of potassium is too small).

3. IRRADIATION FACILITIES FOR PARTIAL I.V.N.A.A.

During all these years devoted to the various developments of I.V.N.A.A. it became evident that the main medical application of these techniques would be to provide data useful for the diagnosis and management of a variety of metabolic bone disorders. However these techniques were not routinely available for clinical applications mainly due to the difficulties encountered in bringing patients to a nuclear center to perform an irradiation with fission reactor neutrons.

It then became obvious that the future developments of I.V.N.A.A. will tend to take place directly in hospitals using in-situ produced neutrons. The choice between the three available irradiation facilities, 14 MeV generator - cyclotron - isotopic sources, depends on severeal factors and more particularly on the kind of technique used : partial or in toto I.V.N.A.A.

The use of local measurements taken on limited portions of the skeleton offers two advantages over in toto measurements : one is dosimetric, the other, technical.

Dosimetrically the sensitivity of the human body to ionising radiations varies enormously from one tissue to another and if the irradiation is focussed on a single fragment of the peripheral skeleton the dose delivered to such radiosensitive organs as the gonads, hematopoietic system or retina will be reduced to a minimum.

Technologically it is all the easier to obtain a homogeneous irradiation and measurement as the volume to be analysed is smaller. Under these conditions the integrated neutron flux necessary will be relatively low, which is a positive factor where the cost of the irradiation device and its biological protection is concerned.

The characteristics of charged particle accelerators are such that the neutron flux supplied is generally more suitable for whole body than for local irradiation. Isotopic sources, are, by far, the most suitable for local activation analysis. The neutron emission of these sources ($\overline{E}_n \leq$ 4 MeV) is adequate for homogeneous irradiation of the superficial bones at low dose levels. The sources are relatively inexpensive and further, require no maintenance.

4. THYROID IODINE CONTENT

In 1967, the estimation of thyroïd iodine was performed in sheep (2,5) by I.V.N.A.A. using fission neutrons obtained in one of the horizontal beam ports of the EL3 reactor in Saclay (thermal neutron flux : 3 x 10^8n $cm^{-2}s^{-1}$). Problems linked to inhomogeneity of irradiation were resolved using ^{129}I as an internal standard. After it is ingested, this isotope can be detected easily in the thyroïd gland by its 28 KeV X-ray emission. The actual amount of the isotope concentrated in the thyroïd can be estimated indirectly by deducting from the administrated known dose the measured urinary excretion. During its exposition to a flux of thermal neutrons ^{129}I is partly converted to ^{130}I and ^{130m}I which emit easily detectable γ-rays. So during I.V.N.A.A. of the thyroïd the ^{129}I acted as a flux monitor allowing an accurate determination of the stable iodine content of the gland (through ^{127}I (n, γ) ^{128}I) and that thought the neutron flux at each point of the thyroïd was not accurately known.

To be able to apply this analytical technique in humans, it was necessary to reduce the radiation dose delivered without reducing too much the sensitivity of detection of iodine. This was done in 1973 by eliminating the γ radiation from the reactor core which was contaminating the irradiation flux with the help of a neutron guide (6). In this device the 0.025 eV neutrons were selected by total reflection on nickel mirrors (the flux used was 1.25 x 10^8 n cm^{-2} s^{-1}). In these conditions, the dose delivered at the surface of the neck (1.5 rem/min) became acceptable.

5. BONE MINERAL CONTENT

From a physiopathological point of view, it seems that in generalized demineralizing bone diseases the variations of calcium content from one bone segment to another are more or less comparable and that these local variations reflect the mineral variation of the whole skeleton. Nevertheless it is obvious that it is preferable to measure bone mineralization on the same patient at various levels of the skeleton.

5.1. Tibia

In 1968, the curved guide of the EL3 reactor was already used to study bone mineral metabolism in man (7). Neutron irradiation was directed against the antero-medial surface of the tibia (irradiation time : 10 to 30 minutes - thermal flux : 2.3 x 10^7 n cm^{-2} s^{-1} - dose absorbed at the level of the skin : 7 rem). Measurement with a 20 cm x 10 cm sodium iodide crystal of the radioactivity induced allows the calculation of the ratios of bone Ca/Na, Ca/Cl and Na/Cl and of the rapidly exchangeable bone sodium.

During this localized irradiation of the tibial region with cold or thermal neutrons, it was possible to detect the prompt γ-rays of phosphorus (77.2 keV) and sodium (90 keV) with a Ge (Li) detector (8)(Fig.1). The sodium, thus detected by two measurements (prompt γ-rays and γ-ray disintegration) could be used as an internal standard to deduce, for the first time in-vivo, the Ca/P mass ratio (1970).

5.2. Hand

5.2.1 Principle. In 1975 we decided to begin our clinical studies on bone metabolism by measuring this Ca/P ratio at the level of the hand (9,10,11).

It is well known that to give rises to γ emitters natural calcium can be irradiated with thermal neutrons, but stable phosphorus has to be transmuted in Aluminium-28 by fast neutrons. Therefore the different behaviour of P and Ca suggested the use of isotopic sources with complementary neutronic characteristics such as 252 Cf, well endowed with slow neutrons, and ^{238}Pu-Be (mean energy 4.5 MeV).

5.2.2. Irradiation. On the basis of these considerations, two irradiation devices were successively designed and built (see Appendix). Two 200 µg ^{252}Cf sources (9.2×10^8 n s^{-1}) and four 10 Ci ^{238}Pu-Be sources (6.8×10^7 n s^{-1}) arranged equidistantly on a ring of diameter 12 cm were placed at the centre of a 1 m paraffin cube. Biological protection was completed by a 1 mm cadmium casing and a 5 cm lead shielding (Dose rate equivalent at the surface of the lead castle < 3 mrem.h^{-1}).

In the first irradiator built in 1975 the sources were fixed and the patient had to place his hand rapidly, in and out of the irradiation position ; this was done by grasping a handle and in pushing it, along rails, to the center of the irradiation ring. This system needed to be improved and in the second device built in 1980, the sources become mobile. When not in use the sources are stored in the back of the paraffin cube. Lucite tubes serve to guide the sources from the storage position to the exposure position. The sources are moved by means of a single pneumatic jack and a remote control motor allows the selection of the sources (^{252}Cf or Pu-Be) before their ejection.

With this new system it is easy to select the nature of the neutrons used for irradiation. Moreover the accuracy in the irradiation time is no longer subordinate to the cooperation or the physical abilities of the patient. To limit irradiation to the hand bones alone, the subject's forearm is protected by a cadmium bracelet.

From measurements carried out on a phantom hand under these conditions, the γ dose rate may be estimated at 0.2 cGy min^{-1} (0.2 rad.min^{-1}) and the mean neutron dose rate at 0.28 cGy min^{-1} (0.28 rad.min^{-1}). For this dose rate (n + γ) of 0.48 cGy min^{-1} (0.48 rad.min^{-1}) the relative biological effectiveness of the neutrons is 5 which leads to a total dose rate of 1.6 cGy equ.min^{-1}. Since the irradiation time is 5 min the absorbed dose is 8 cGy equivalent (8 rads equivalent). The dose equivalent delivered to the whole body is then about 0.035 mSv (2.5 mrem) (12).

5.2.3. Measurement of induced radioactivity. One minute after the irradiation is over, the activity induced in the subject's hand is measured with two low-background sodium iodide scintillators (12.7 cm x 12.7 cm), placed in a 10 cm low activity lead shield. Each detector is connected to a stabilised amplification line and the total γ activity spectrum is recorded between 1.10 MeV and 3.50 MeV by a multi channel analyser linked to a computer.

5.2.4. Ca/P ratio. When both kinds of neutron sources are used simultaneously the four major elements of the bone mineral matrix are measured : Ca (^{49}Ca), Na (^{24}Na), Cl (^{38}Cl) and P (^{28}Al). If the measured calcium comes essentially from bone tissue, it is clear that the phosphorus results obtained cover not only the bone phosphorus but also that of the soft tissues (in the hand 5 to 7 % of the total phosphorus). Allowing to this unavoidable inaccuracy, the bone Ca/P ratio so measured in vivo (mean value on 55 subjects : 1.96 ± 0.17) corresponds well to the value 2.18 recommended in the literature (9, 10, 11).

5.2.5. Calcium. With ^{252}Cf sources alone, the only elements likely to be measured are Ca and Na (and Cl).

Nowadays, serial measurements of hand bone calcium concentration are routinely taken on patients to determine the course of their diseases (osteoporosis or renal diseases) and the response to the therapeutic treatment.

5.2.6. Sodium. From a clinical point of view the measurement of bone sodium content also appears to be potentially usefull, essentially through kinetic studies. With this in mind, the ^{24}Na radioactivity variations measured on 14 subjects were plotted against time, and each curve fitted to a function being the sum of two exponentials (19).

Two pools were identified : the first corresponding to a fast turnover, with a half-life of about one hour appears to represent the extracellular fluid sodium ; the other with a slower turnover process with a half-life averaging 79 hours, if the calcium concentration of the hand bones is normal, and 35 hours if this calcium content is sub-normal. This more slowly exchangeable sodium appears to represent the bone sodium. The mean ratio of calcium to slowly exchangeable sodium is estimated at 47.7, and that of quickly exchangeable sodium to slowly exchangeable sodium at 0.65. These various parameters should be of interest in bone pathology because a decrease in bone calcium go with a parallel decrease in bone sodium and an accelerated bone sodium turnover.

5.3. Spine

Once the in vivo Ca measurement at the level of the hand became a clinical routine examination we recently decided in 1980 to carry on our studies on bone metabolism by measuring the mineral content at the vertebral column which contains a large amount of trabecular bone, where metabolic activity is known to be high and where osteoporotic damage is supposed to be detectable earlier.

As the thickness of the trunk renders bilateral irradiation not very useful , an acceptable unilateral irradiation system was developed.

A 100 µg ^{252}Cf source, housed in a shielded cylinder, is transferred pneumatically via polyethylene tubes to the operating position. The irradiation is done with a source to skin distance of 2.7 cm (2 cm of lucite as premoderator), with no reflector or cadmium sheet. During the 10 min irradiation the patient lies face-down on a movable bed ; the absorbed dose is 12 cGy equivalent to the skin and 3 cGy equivalent to the spine (on 7 vertebrae). The induced activity is measured with a 15 cm x 15 cm NaI crystal which is placed against the irradiated vertebrae. Before the irradiation a thin strip of gold is stuck on the skin of the patient, along the spine, and used as an external standard to normalize the results. The statistical accuracy of patient measurements is 1.8 % and the reproducibility (precision) measured in vitro on a spine phantom is 2.2 %. The in vivo reproducibility is actually being established from repeated measurements on 10 patients. Preliminary results show that the precision on the determination of spinal calcium could be better than 3 % using our external standard technique.

Calcium results are normalized according to the hand bone volume calculated from the projected hand bone area. This area is measured by planimetry on a dorso-palmar radiographic image of the hand bones.

Measurements of calcium content in the hand bones are obtained with satisfactory precision ($< \pm 2$ %), sensitivity (200 mg) and accuracy (statistical accuracy : ± 1 % - accuracy on standards $< \pm 2.7$ %).

The first clinical study covered more than 150 reference subjects (13), men and women, between 20 and 100 years of age (fig. 2). The total measured bone calcium varies between 4.5 and 16 g and the bone calcium concentration between 0.095 and 0.25 g of calcium per cubic centimetre of bone. In these controls, the average calcium concentration per decade does not change significantly between the age of 20 and 60 in women or 70 in men. The diminishing calcium concentration observed in the hand bones with advancing age (after 60 in women and 70 in men) occurs probably through bone atrophy. In post-menopausal women, this bone mineral loss follows an exponential law - $(Ca) = 0.34\ e^{-0.013\ (years)}$ ($r = 0.99$) - corresponding to an annual calcium loss of 1.3 %.

The second group studied numbered about 114 patients (73 women and 41 men), all over 40 and suffering from primitive osteoporosis confirmed radiologically by collapse of at least one vertebra (14, 15) (Fig. 3, 4). Bone calcium concentration is found to be significantly lower than that of the control subjects in most of men, in 93 % of osteoporotic women aged between 50 and 59, in 66 % between 60 and 69 and only 5 % over 70.

The third group consisted of 46 uremic patients dialysed for at least two years (16, 17, 18). All the patients with radiological signs of hyperparathyroïdism have a very low hand calcium concentration. Amongst the patients without signs of hyperparathyroïdism, 25 % have, nevertheless, a calcium concentration significantly lower than that of controls.

The correlation between the hand bone calcium mass and the radial epiphysis bone mineral content (B.M.C.) in the reference group is high ($r = 0.87$ $p < 0.001$). This correlation is very slightly smaller ($r = 0.85$ $p < 0.001$) in osteoporotics but significantly smaller ($r = 0.72$ $p < 0.001$) in uremics.

Correlations between calcium mass of the hand and histomorphometric parameters measuring bone atrophy (trabecular bone volume, trabecular bone volume minus trabecular osteoïd volume) are not significant in osteoporotics and uremics. In uremics it is interesting to note a significant negative correlation with an histological index of iliac bone resorption (number of osteoclasts per mm^3 of trabeculae, $r = 0.495$ $p < 0.05$).

Finally in osteoporotics weak but significant correlations have been found between hand bone calcium concentration and metacarpal index ($r = 0.55$ $p < 0.01$) or number of thoracic vertebral fractures ($r = -0.41$ $p < 0.05$).

As it seems very difficult to estimate the volume of bone irradiated, this technique should be used only on longitudinal studies to follow the spinal calcium variations in the same patient.

6. FUTURE WORK

In the near future, we will direct our efforts towards this spinal calcium measurement. In fact we should like to compare, on the same patient, the evolution of the calcium mass in cortical (hand) and trabecular (vertebra) bone. These localized bone calcium measurements ought to give an accurate picture of the progress of bone mineral loss in each individual suffering from pathological disorders characterized by osteopenia and above all to give a precise idea of the effectiveness of treatments aimed at slowing down or eliminating these conditions.

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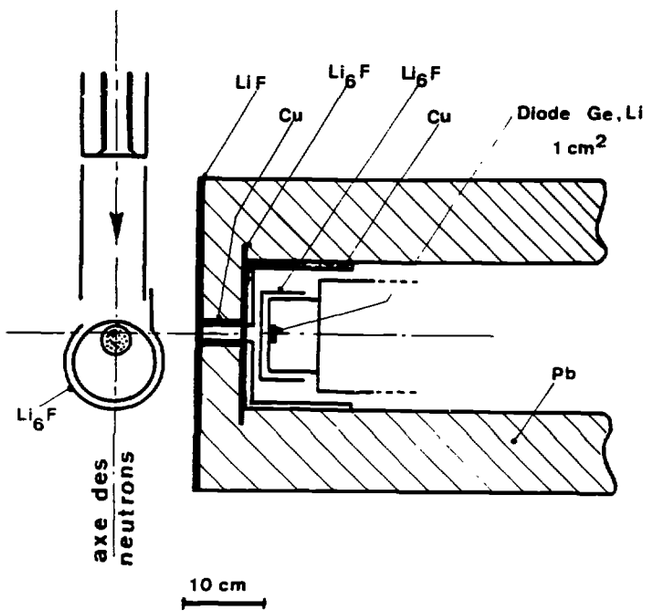

LiF
Cu
Li_6F
Li_6F
Cu
Diode Ge, Li
1 cm²
Pb
Li_6F
axe des neutrons
10 cm

FIG. 1

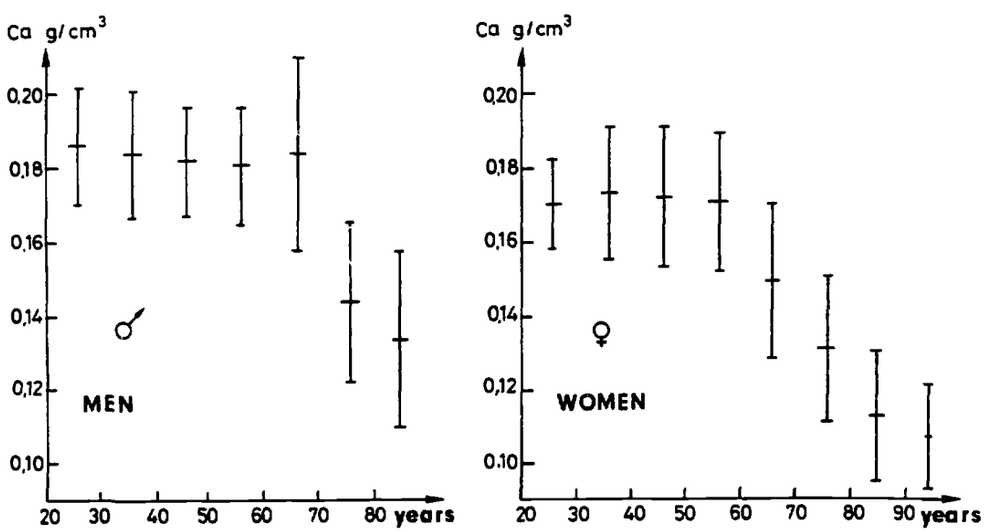
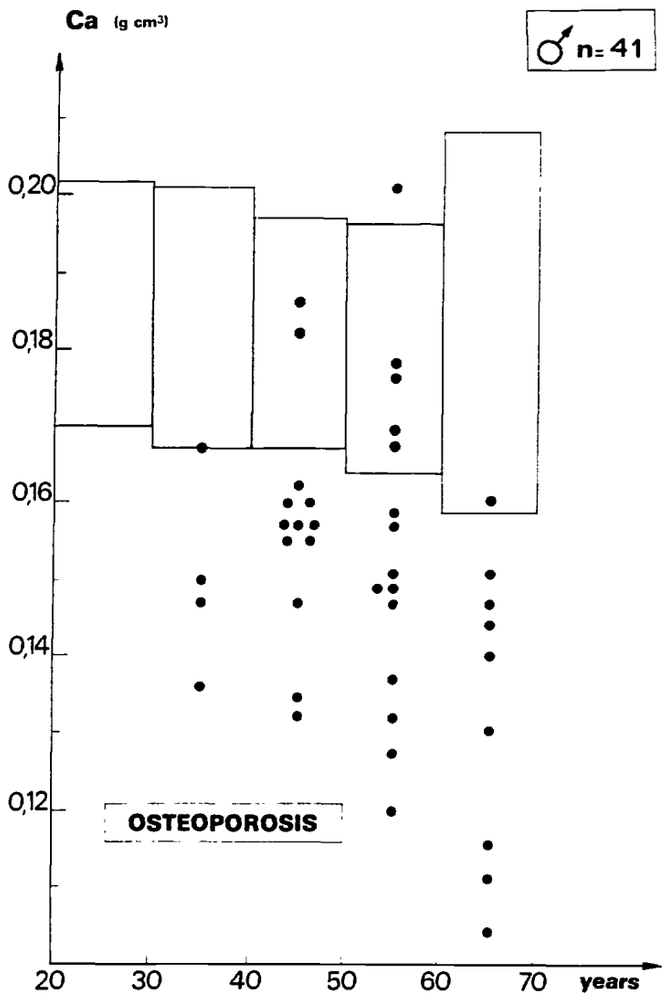

Ca g/cm³
0,20
0,18
0,16
0,14
0,12
0,10
MEN
20 30 40 50 60 70 80 years
Ca g/cm³
0,20
0,18
0,16
0,14
0,12
0,10
WOMEN
20 30 40 50 60 70 80 90 years

FIG. 2


Ca (g cm3)
♂ n= 41
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0,16
0,14
0,12
OSTEOPOROSIS
20
30
40
50
60
70
years

FIG. 3

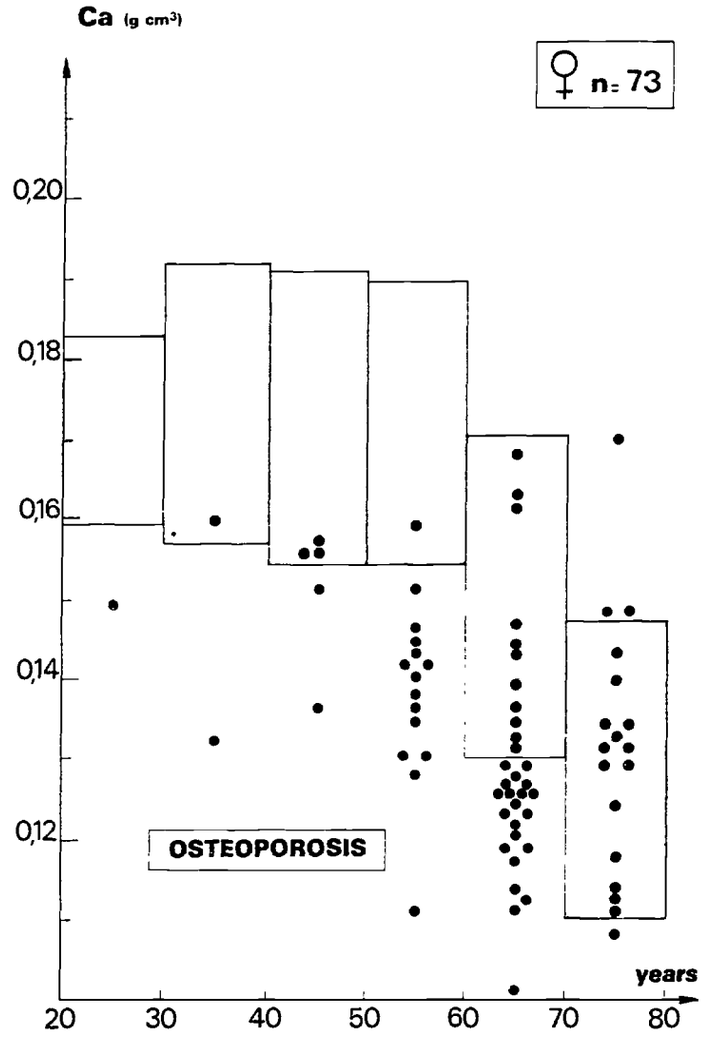
Ca (g cm³)
♀ n= 73
0,20
0,18
0,16
0,14
0,12
OSTEOPOROSIS
years
20
30
40
50
60
70
80

FIG. 4