KINETICS OF ^{47}Ca IN MAN

V. Jovanović, S. Popović, I. Latković
and I. Šimonović

*The Institute for Medical Research incorporating the Institute of Industrial Hygiene,
Yugoslav Academy of Sciences and Arts, and Department of Medicine,
Medical Faculty, University of Zagreb, Zagreb*

Kinetics of ^{47}Ca was investigated in 9 healthy subjects. In this paper slight modifications of the Wenderberg method were applied. Our modification of the method is technically simpler, giving equally valuable results. The following values were obtained: Accretion: 0.57 ± 0.12 g Ca/day, S compartment: 2.10 ± 0.32 g Ca and E compartment: 2.86 ± 0.83 g Ca.

Calcium is one of the most important body minerals. A whole series of physiological processes depend on the stability of its level in body fluids. In the regulation of body calcium metabolism and the maintenance of the constant level of its concentration in body fluids a great many humoral factors take part, so that the metabolism of body calcium in normal conditions represents a complicated system of dynamic equilibrium (1). The study of the disturbed body calcium metabolism is interesting in the first place in connection with bone lesions. On other hand, the study of the absorption and excretion of calcium is of importance in a number of disorders of the gastrointestinal tract and the kidney. Furthermore, the investigations into the metabolism of body calcium are valuable in dealing with endocrinopathies, especially with hyper- and hypoparathyroidism, hyperthyreosis, and some other endocrinological disorders (2, 3).

Classical chemical methods and relative measurements are of very limited value in the study of calcium metabolism. Only by the use of radioactive isotopes have conditions been created for kinetic analysis. The introduction of radioactive isotopes of calcium and of its metabolic homologue strontium has allowed more detailed studies of the dynamic aspects of calcium metabolism.

Kinetic investigations aim at describing the behaviour of radioactive tracers by means of adequate mathematical expressions. Mathematical

expressions formulate the size of the compartments in which the tracers are distributed as well as the speed of communication between individual compartments. Kinetic models are set up on the basis of the knowledge of physiological processes, so that the parameters obtained may correlate with physiological processes. In this way, by means of tracers, it is possible to follow dynamic changes in the course of physiological and pathological metabolic processes.

Radioactive calcium in the organism, in the course of time, achieves an equilibrium with stable calcium and is quickly distributed in the extracellular and intracellular compartments. Part of calcium goes into soft tissues. At the same time radioactive calcium is incorporated into the skeleton where new bones are formed, in the regions of osteoclasia, and canalicular and lacunar surfaces (4). Radioactive calcium accumulates in bone crystals on the basis of physico-chemical processes (5): by diffusion in the hidration shell, by ionic exchange at the crystal surface, and by intracrystalline exchange. From the physiological point of view these processes mean the incorporation of calcium into the bone that is being formed, a quick exchange of calcium with a small portion of the bones located near the periphery of the vascular system, and a slow exchange of calcium with some deeper mineral fractions in fully calcified bones (6).

According to the equilibration rate, that is the disappearance of calcium from the plasma, the distribution space of radioactive calcium can be divided into two pools: one acquiring the equilibrium quickly and one achieving this state more slowly. On the basis of infusion studies with ^{45}Ca *Rich* (7) has concluded that the quick pool achieves the equilibrium with the plasmatic one within 15 minutes. *Bauer* and *Ray* (8) and *Aubert* and *Milhaud* (9), on the basis of their studies, have formulated a kinetic model of the metabolism of body calcium with four compartments. The computer analysis of this model has shown that three of its four compartments reach the equilibrium comparatively quickly (up till one hour). The size of this »quickly« exchangeable compartment amounts to about one third to one half of the total calcium pool. Most authors agree that the plasma, extracellular fluid, and part of the intracellular fluid belong to this pool. According to some data, part of the exchangeable bone calcium also belongs to this quick pool (10). What has also been found is that the size of the quick pool varies with the size of the exchangeable bone calcium (11).

The equilibration of the slowly exchangeable pool may take up as long as to 3 days. The size of this pool amounts to about a half of the total calcium pool. The slowly exchangeable pool is connected with the exchangeable bone calcium, yet the whole of the latter cannot be ascribed to this pool, because first, part of the exchangeable bone calcium belongs to the quickly exhangeable pool and, secondly, because this slowly

exchangeable calcium pool also contains part of the non-bone calcium which equilibrates slowly. According to *Heaney* (12), this in the first place relates to the cartilage.

The above quoted data give the basis for the setting up of kinetic models of mineral bone metabolism. A series of details from this field, not being touched upon in this paper, could be found in the reviews of calcium kinetics (13).

The basic parameter obtained by kinetic analysis is the calcium deposit rate in the bones expressed in g Ca/day. The symbols used for this parameter in kinetic studies vary according to the authors: V_{0+}, (9), BFR (bone formation rate) (14), a (accretion) (8). We have adopted the latter term.

According to *Wendeberg* (15), the kinetics of radioactive calcium can adequately be presented by an open two-compartment model drained by excretion and accretion (Fig. 1).
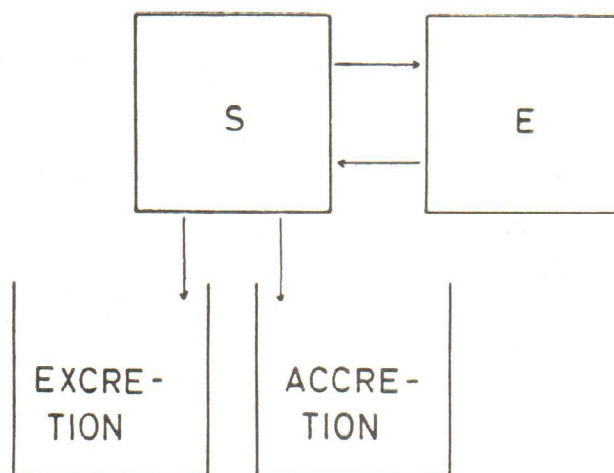

Fig. 1. *Wenderberg's two-compartment model of calcium and strontium kinetics*

The basic equation for the calculation of pools by means of this model is:

$$S + E = \left(Ret_{t_{equ}} - a \int_{t_0}^{t_{equ}} s^*(t)\, dt \right) / s^*_{t_{equ}} \tag{1}$$

S denotes the size of the quickly exchangeable pool, and E the size of the slowly exchangeable pool. Both compartments are expressed in g Ca.

Ret is the retention of the radioactive isotope expressed in the percentage of the dose.

$s^*(t)$: the function showing changes in the specific activity of the plasma in relation to time. The specific activity of the plasma is expressed in the percentage of the dose/g Ca.

a: accretion expressed in g Ca/day.

t_{equ}: the time when specific activities in both S and E compartments (s^* and e^*) are equal.

The expression (1) and additional expressions (2), (4), and (8) are in accord with mathematical deductions for the two- compartment model. These expressions are based on three assumptions made on experimental grounds:

(1) The size of the S compartment is obtained by dividing body retention one hour following injection by the plasma specific activity obtained at the same time. Thus, this is the quickly exhangeable pool, the anatomical location of which has already been described above.

(2) On the basis of his model and external measurements in the region of the knee (15), *Wenderberg* has assumed that at a certain time (t_{equ}) specific activities in S and E compartments are equal. The computer analysis of the curve of the plasma specific activity in 5 normal persons (8) shows the value of the t_{equ} to be 24 hours.

(3) Specific activities of S and E compartments one day following the time of equilibration have a constant relation: $s^*/e^* =$ const. The time limitation of this relation is 7–10 days following the isotope injection. After that time the radioactive calcium incorporated into the bones begins to return into the S compartment. Opinions on the cause of this return are divided. *Bauer* (16) and *Heaney* (12) think that at that time there occurs resorption of the part of the bones in which calcium was incorporated, while *Nordin* (17) is of the opinion that some recrystallization-exchangeable processes are involved.

Wendeberg's model has been made by a simplification of the four compartment models. As regards accretion, this model is just as valuable as the four-compartment models. By giving a detailed description of *Wendeberg's* model, we are far from claiming that this is the only right way in tackling the problems of calcium kinetics. For routine investigations *Wendeberg's* model has advantages owing to its simplicity and a comparatively short time it takes up for testing. The calculation of calcium kinetic parameters according to *Wendeberg's* model has been applied by a great many authors (18, 19, 20), and at present this is the most frequently used method. Moreover, this test is suitable for the analysis of external measurements by bone-seeking isotopes. Having in mind all these specificities of the *Wendeberg* model, this model has been used in the first stage of our studies of radioactive calcium. In contrast to the original procedure, our investigations have been reduced to seven days.

METHODS AND SUBJECTS

In this study the kinetics of body calcium was investigated in 9 healthy subjects chosen according to the following criteria:
- age over 25
- absence of serious and generalized disorders
- absence of osteoporosis
- normal serum calcium, phosphorus, and alkaline phosphatase values.

The dose applied was 20–25 μC ^{47}Ca to which 0.5 mg of stable calcium was added in the form of $CaCl_2$. The dose was applied intrave-nously. The subjects were observed for 7 days. The first day the blood was sampled 1/4, 2, 6, and 12 hours following injection, and the fol-lowing days, including the 7th one, one blood sample a day was col-lected. In the course of the experiment, the activity of excreted calcium in the urine and feces was determined for each day separately.

The activity of blood and urine samples was measured by the »well« type scintillation counter, made by the firm »Nuclear Chicago«, with the 2" crystal diameter, and by the amplitude analyser of the same firm. The measurement of the activity of the samples in different inter-vals and the knowledge of the average value of the concentration of stable calcium in the blood allowed the calculation of the percentage
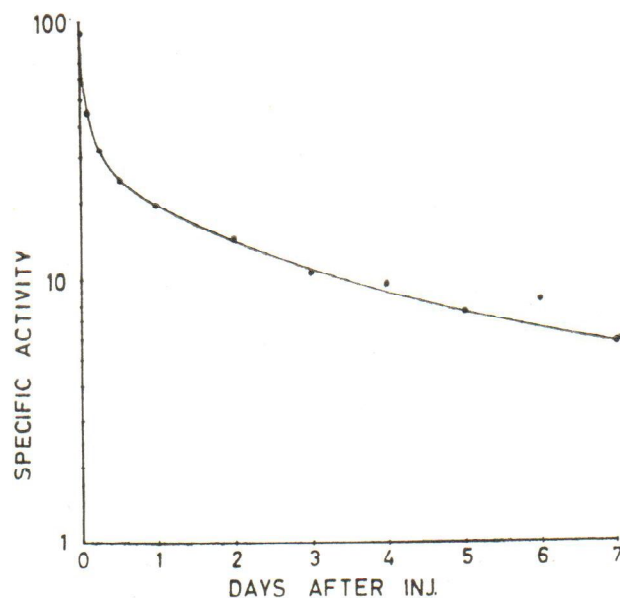

Fig. 2. *The curve of the ^{47}Ca specific activity (% of the dose/g Ca) in the plasma (a semilogarithmic presentation)*

of the injected dose per gram of stable calcium for each plasma sample. In this way the curve of the plasma specific activity as the function of time was obtained (Figures 2 and 3). The activity excreted in the urine
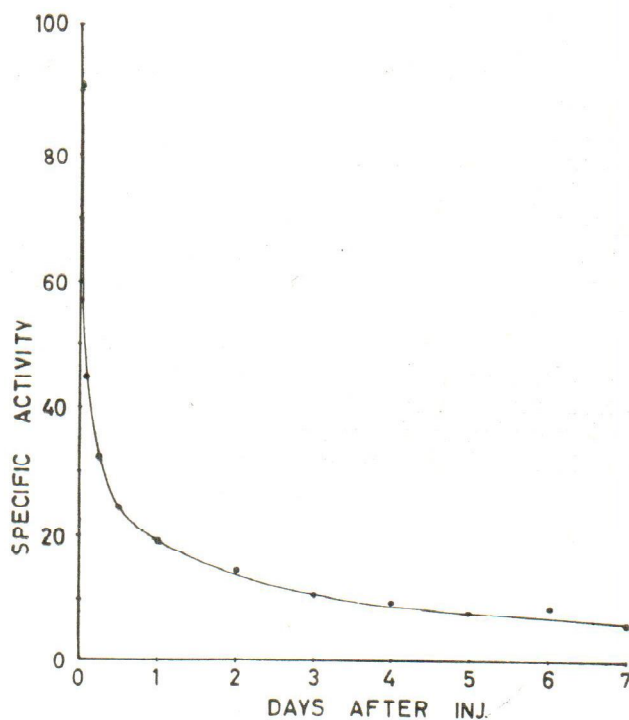

Fig. 3. *The curve of the ⁴⁷Ca specific activity (⁰/₀ of the dose/g Ca) in the plasma (a linear presentation)*

was determined by the measurement of the aliquot of the urine in the scintillation counter and by recalculating it to the total urine volume excreted. Feces measurements were made by a special equipment »To-bor« (Nuclear Chicago) consisting of two scintillation detectors facing each other, both connected to a one-channel impulse amplitude analyser. The curve of calcium body retention as a function of time was obtained by a daily reduction of the activity excreted in the urine and feces from the dose given (Fig. 4). The values for accretion and »exchangeable« calcium compartments were obtained, by the Wendeberg method (15), from the curves of the plasma specific activities and the curve of the calcium retention in the organism.
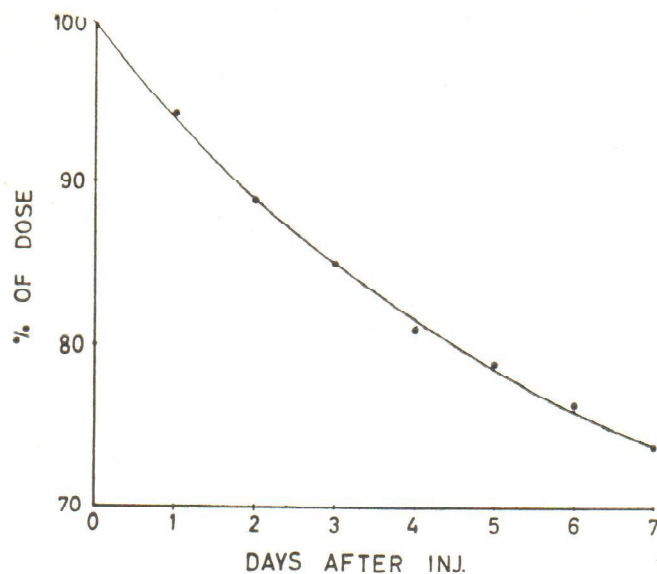
Fig. 4. *The curve of the ^{47}Ca body retention*

The calculation itself will be illustrated by one of the cases studied. On the basis of *Wendeberg's* model, body retention in two intervals after the isotope injection $(t_0 — t_1)$ and $(t_0 — t_2)$ can be expressed as follows:

$$Ret_{t_1} = S \times s^*_{t_1} + E \times e^*_{t_1} + a \int_{t_0}^{t_1} s^*(t)\,dt \qquad (2)$$

$$Ret_{t_2} = S \times s^*_{t_2} + E \times e^*_{t_2} + a \int_{t_0}^{t_2} s^*(t)\,dt \qquad (3)$$

where

S and E = the size of compartments
s^* and e^* = specific activities in S and E compartments
a = the accretion rate

Thus, the first term on the right side of the above quoted equations denotes the total activity in the S compartment at a certain time, the second term the total activity in the E compartment, and the third term the accumulation of the activity deep in the bones for the same time

interval. If the fall of the isotope concentration in the serum during the $t_1 — t_2$ interval represents the fall of the isotope in the whole exhangeable compartment, then the relation reads:

$$s^*_{t_1} / e^*_{t_1} = s^*_{t_2} / e^*_{t_2} = k \qquad (4)$$

The equations (2) and (3) can now be presented in a simpler form:

$$Ret_{t_1} = s^*_{t_1} Q + a \int_{t_o}^{t_1} s^* (t) \, dt \qquad (5)$$

$$Ret_{t_2} = s^*_{t_2} Q + a \int_{t_o}^{t_2} s^* (t) \, dt \qquad (6)$$

where $Q = S + \dfrac{E}{k}$

From the retention curve of this subject (Fig. 3) it can be read:

$$Ret_{t_1} = 84.9\%, \qquad Ret_{t_2} = 76.0\%$$

and the curve of the plasma specific activity (Fig. 2 and 3) shows specific activities in $t_1 — t_2$:

$$s_{t_1} = 11.02\%/g \ Ca, \qquad s_{t_2} = 6.76\%/g \ Ca$$

The integrals from equations (3) and (4) are obtained graphically by determining the surface enclosed by the curve of the plasma specific activity and the abscissa between the ordinates t_0 and t_1 and t_0 and t_2 respectively. In the case presented the values of these intervals are as follows:

$$\int_{t_o}^{t_1} s^* (t) \, dt = 58.8 \frac{day \times \%}{g}, \qquad \int_{t_o}^{t_2} s^* (t) \, dt = 84.9 \frac{day \times \%}{g}$$

Or:

$$84.87 = 11.02 \ Q + a \ 58.8$$

$$76.00 = 6.76 \ Q + a \ 84.9$$

Thus:

a = 0.49 g/day

a = 7.64 mg Ca/kg body weight

The size of the S compartment is obtained by dividing body retention one hour following injection by the isotope concentration one hour following injection:

$$S = \frac{Ret_{1 \text{ hour}}}{s^* \text{ (1 hour)}} = \frac{99.73}{63.4} = 1.57 \text{ g Ca} \tag{8}$$

that is: S = 24.5 mg Ca/kg body weight.

About 24 hours following injection there exists a transient equilibrium between S and E compartments. This means that specific activities at that time are equal, and the total exchangeable calcium compartment can be calculated: S + E.

$$S + E = \left(Ret_{t_{equ}} - a \int_{t_o}^{t_{equ}} s^* (t) \, dt \right) / s^*_{t_{equ}} \tag{1}$$

To apply it to the case presented, the equation reads:

$$Ret_{t_{equ}} = 93.80\%, \quad s^*_{t_{equ}} = 19.24\%/g, \quad \int_{t_o}^{t_{equ}} s^* (t) \, dt = 29.2 \frac{day \times \%}{g}$$

That is:

$$S + E = (93.8 - 0.49 \times 29.2) / 19.24$$

or:

$$S + E = 4.13 \text{ g Ca}$$

or:

$$S + E = 64.6 \text{ mg Ca / kg body weight}$$

$$E = 4.13 - 1.57 = 2.56 \text{ g Ca}$$

$$E = 40.0 \text{ mg Ca / kg body weight}$$

RESULTS AND DISCUSSION

In the studies carried out so far 9 healthy subjects were observed. The results, shown in Table 1, are in good agreement with data reported by Wendeberg. In normal subjects accretion is about 0.5 g Ca/day, and the E + S compartment 4–6 g Ca (15). From our data it can be seen that the standard deviations of the parameters analysed are within 30% at the maximum. Compared with literature data (Table 2) our values are somewhat higher than those obtained by other authors (21).

In our calculations the time intervals of 3 and 6 days while in the

Table 1

Subject	% of the dose excreted in the urine through 7 days	% of the dose excreted in the feces through 7 days	Compartment S g	Compartment S mg/kg	Compartment E g	Compartment E mg/kg	Accretion g	Accretion mg/kg	Calcium in plasma mg %
1. K. R. ♂ 37 y.	20.12	5.97	1.57	24.5	2.56	40.0	0.49	7.64	9.6
2. K. I. ♂ 45 y.	21.88	10.66	1.87	26.0	3.06	42.5	0.38	5.29	9.0
3. M. S. ♂ 38 y.	12.82	17.10	2.03	36.2	2.52	45.0	0.40	7.08	11.4
4. T. K. ♂ 62 y.	5.05	8.14	2.32	25.8	3.68	40.9	0.59	6.61	10.0
5. P. B. ♂ 25 y.	13.31	8.9	2.11	29.4	2.72	37.8	0.61	8.50	9.4
6. M. S. ♂ 48 y.	15.08	12.9	2.54	26.5	3.11	32.4	0.54	5.68	10.0
7. Č. N. ♂ 30 y.	24.23	6.1	1.74	25.6	2.62	38.5	0.81	11.9	9.6
8. Č. I. ♂ 35 y.	19.26	8.9	2.34	33.5	2.44	34.8	0.60	8.58	10.8
9. A. V. ♂ 26 y.	12.79	8.22	2.36	30.1	3.08	39.2	0.69	8.8	9.8
Mean	16.06 ± 5.90	9.65 ± 3.51	2.10 ± 0.32	28.3 ± 6.1	2.86 ± 0.89	39.0 ± 3.81	0.57 ± 0.12	7.79 ± 1.99	9.95 ± 0.74

Table 2

	a (g Ca/day)
Lafferty et al.	0.494
Dymling et al.	0.359
Nordin et al.	0.369
Heaney	0.398
Our results	0.570

original test those of 5 and 10 days were used. In this way we have reduced the duration of the experiment and with much certainty avoided the periods when radioactive calcium that was incorporated in the bones re-enters the plasma. This process begins 7–10 days after injection.

However, this calculation made in the early phase gives increased accretion values. *Nordin* (22) has hown that mathematically obtained accretion values go down with time, which depends on the time interval taken into account. *Bauer* (23) has emphasized that lower values cannot by any means be considered more correct, because at the time they relate to the isotope return from the bones. In view of all this, accretion represents the index of the skeletal formation rate. If the same time intervals are employed for calculations, the values obtained in this way appear to give a real picture of changes in the skeletal formation rate. Somewhat increased accretion values may also have been due to the choice of subjects. They were all men with a relatively larger skeletal mass. According to the definition, the accretion rate is the rate constant related to the turnover of the skeletal mass. This means that the larger the skeletal mass the higher the accretion rate, and vice versa.

From the model established in this way and the calculated accretion value it is not possible to assess the calcium balance which represents the difference between the skeletal formation and skeletal resorption rate.

The physiological significance of calcium compartments is stressed in the introductory part of this paper where it is also pointed out that to give a detailed anatomical characteristic of these compartments is not an easy task.

The relation between urinary and endogenous fecal calcium (the relation between the dose excreted in the urine and that excreted in the feces) in our subjects was 1.7, which is in fairly good agreement with the relation 1.3 given by *Bronner* (24). Here possible variations in this

relation should be kept in mind, for in the aged, from 50–70 years old, excretion for the most part occurs through feces (25); moreover, in some endocrinopathies (hyper-hypo-parathyroidism) there are considerable changes in the endogenous foecal calcium values (26).

CONCLUSION

Kinetic analysis of ^{47}Ca gives valuable information for the study of calcium metbolism.

In this paper slight modifications of the well-known Wendeberg method were applied with satisfactory results.

The most valuable data obtained are those relating to calcium accretion in the skeleton. Though the absolute accretion rate depends to a certain extent on the method applied in the study, this parameter, when using the same method, is, none the less, a reproducible value and can be regarded at least as a useful index.

The kinetic analysis of ^{47}Ca also allows the calculation of calcium body compartments, but the meaning of these data remains obscure.

Owing to the modified method and perhaps to the choice of subjects, the results of the kinetic analysis of ^{47}Ca in healthy persons obtained in this study only partly agree with the results of other authors.

In our opinion, our modification of the method is technically simpler, giving equally valuable results.

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Sadržaj

KINETIKA RADIOAKTIVNOG KALCIJA (⁴⁷Ca) KOD ČOVJEKA

U devet zdravih osoba ispitivana je kinetika radioaktivnog kalcija. U svom radu primijenjene su manje modifikacije Wendebergove metode. Te modifikacije pojednostavljaju ispitivanja, a daju jednako vrijedne rezultate. Dobivene su ove vrijednosti: akrecija: 0.57 ± 0.12 g Ca/dan, S prostor: 2.10 ± 0.32 g Ca i E prostor: 2.86 ± 0.83 g Ca.

Institut za medicinska istraživanja
i medicinu J. A. Z. U.
i Interna klinika Medicinskog fakulteta.
Sveučilišta, Zagreb