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CALCIUM METABOLISM EVALUATED BY ^{47}Ca KINETICS

Methodologic aspects with optimization of a whole-body counter

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Abstract

Optimization of a four-crystal stretcher geometry whole-body (WB) counter was performed. The measured geometric characteristic did not differ significantly from the theoretically calculated characteristic ($p>0.05$). The linearity of the WB counter was high in the range 3.7 kBq to 3.7 MBq. Longtime variability of local background was CV=3.7 per cent. In vivo sensitivity was calculated to 18 cpm/kBq and the detection limit to about 5 kBq. The reproducibility was estimated to 0.7 per cent. The WB counter was used in ^{47}Ca turnover and calcium balance studies of 15 normal individuals. The data were analysed according to a modification of the expanding calcium pool model using an improved Bauer-Carlsson-Lindquist formulation. Variability and method errors of main parameters for calcium metabolism were evaluated.

Calcium tracer atoms introduced into the living organism are distributed and excreted through the same pathways and processes as ordinary body calcium and therefore provide an efficient tool for studying calcium homeostasis.

Experimental findings have led to model systems for tracer calcium distribution consisting of one or usually more exchangeable pools from where irreversible losses of tracer take place to mineralized bone, by renal and faecal excretions and dermal losses (2, 4, 6, 8, 11, 13, 16, 18).

Two methods have been used to analyse the calcium tracer data. The first method known as the Bauer-Carlsson-Lindquist (BCL) formulation (3) states that

$$R(t) = M \times S^*(t) + m \times \int_0^t S^*(t)\,dt \qquad (1)$$

where R(t) is whole-body retention, M exchangeable calcium pool size, m bone mineralization rate, and S*(t) serum calcium specific activity. The second method, the turnover difference (TD) formulation (9), expresses that

$$t_r = u + e + m \qquad (2)$$

where u is renal calcium excretion rate, e endogenous faecal calcium excretion rate, m bone mineralization rate, and t_r pool turnover rate equivalent with change in serum calcium specific activity.

The BCL formulation is based on whole-body retention measurements and therefore yields mineralization rate estimates, which are not influenced by insufficient collection of faeces and urine or by unknown variations in dermal losses. Furthermore, the dermal calcium loss, which is of importance for the overall calcium balance, may be derived from measured whole-body retention curves and the cumulated renal and faecal excretions of tracer (13). Consequently, it appears that an accurate and sensi-

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tive whole-body (WB) counter is of importance for any calcium tracer turnover study.

The aim of the present report is to describe the optimization of a medium sensitive 4 crystal WB counter with stretcher geometry. The WB counter was used in ^{47}Ca turnover and calcium balance studies based on the continuously expanding calcium pool model (4) with individual corrections for faecal lag time and estimation of dermal calcium loss.

Material and Methods

Experimental subjects. Fifteen normal subjects (12 women and 3 men) aged 24 to 65 years (mean 45 years) were investigated. The subjects were without medication or any evidence of bone disease.

Ethics. Informed consent (Helsinki declaration II) was obtained from all the normal volunteers.

Calcium balance technique. During the 7 days study and one week before (equilibration period), the subjects consumed a diet with a composition close to their daily fare. During the investigation, the serving was doubled, one for the person, one for analysis. Daily faecal and urine collections were made. Carmine red was given orally as a visual faecal marker. ^{51}Cr tablets were given 3 times a day with the meals, as a non-absorbable faecal marker. After homogenization, duplicate samples of daily diet and faeces were ashed. The calcium content was determined by atomic absorption spectrophotometry. The faecal ^{51}Cr content was used to correct the faecal calcium output.

^{47}Ca kinetics. After the 7 days equilibration, 0.74 MBq (20 μCi) of $^{47}CaCl_2$ was injected intravenously. Blood samples were drawn frequently the first 6 h and once daily for the next 7 days. The ^{47}Ca content was determined in serum samples and in daily urine and faecal samples using a scintillation well counter. Further, a whole-body ^{47}Ca retention determination was performed twice a day by an intermediate sensitive four-crystal stretcher geometry WB counter. All measurements on the WB counter were performed from both the front and the back to eliminate the effect of redistribution. The mean value of these measurements was used.

Mathematical model

Abbreviations for the tracer model:

Q_0 = activity of intravenously injected isotope (cpm)
$Q(t)$ = fraction of activity injected in the expanding pool
$R(t)$ = whole-body retention (fraction of activity injected)
$M_1(t)$ = the expanding calcium pool mass at t=1 day (mmol Ca)
$S^*(t)$ = serum specific activity (fraction of activity injected per mol stable calcium)
$BM(t)$ = activity incorporated into bone by mineralization as a fraction of activity injected
$E(t)$ = fraction of activity injected retained in the bowel
$F^*(t)$ = cumulated faecal activity (fraction of activity injected)
m = mineralization rate (mmol Ca/day)
l = total urine, dermal and faecal excretion rate (mmol Ca/day)
u = urine excretion rate (mmol Ca/day)
e = endogenous faecal calcium excretion rate (mmol Ca/day)
d = dermal excretion rate (mmol Ca/day)
λ = rate constant (1/day)
t_r = turnover rate (mmol Ca/day)
r = resorption rate (mmol Ca/day)
f = faecal excretion rate (mmol Ca/day)
Δt = time interval for lag of activity in bowel (days)
A = positive constant (1/mmol Ca)
b = positive constant
β = net absorption of calcium (per cent of intake)
k = dietary intake rate (mmol Ca/day)

The ^{47}Ca kinetic data were analysed according to a further development of the continuously expanding exchangeable calcium pool model proposed by BURKINSHAW et coll. (4). The model is composed of (Fig. 1): 1) The expanding calcium pool mass (M(t)) where the activity (Q_0) is initiated. From this pool, the activity is irreversibly lost to 2) bone (BM(t)) by active mineralization (m), 3) bowel (E(t)) where the activity after a lag time (Δt) is excreted in faeces (e), 4) urine (u), and 5) sweat and other dermal losses (d).

Serum specific activity ($S^*(t)$) has been shown, in the first hours after injection of the tracer, to vary as a power function because of dominating pool expansion. If this expansion is assumed to apply in the full course of the study and no isotope from mineralized bone is supposed to return to the pool because of bone resorption, one can deduce (13):

$$S^*(t) = A \times t^{-b} \times \exp(-\lambda \times t^{(1-b)}) \quad (3)$$

where A, b and λ are constants estimated from a curve-fitting procedure.

The bone mineralization rate was found using the BCL formulation modified by the individual lag of activity in the bowel and dermal calcium loss through:

$$R(t) = Q(t) + BM(t) + E(t) \quad (4)$$

By substitution and integration it was shown (13) that retention R(t) was given by

$$R(t) = m \times A_1 + (u+d) \times A_1 \times \exp(-\lambda \times t^{(1-b)}) + (1 - m \times A_1 - (u+d) \times A_1) \times \exp(-\lambda \times (t-\Delta t)^{(1-b)}) \quad (5)$$

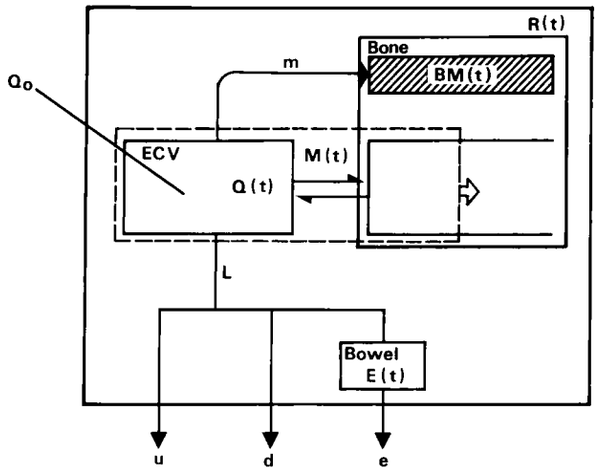

Fig. 1. The expanding calcium pool model modified from BURKINSHAW et coll. (4).

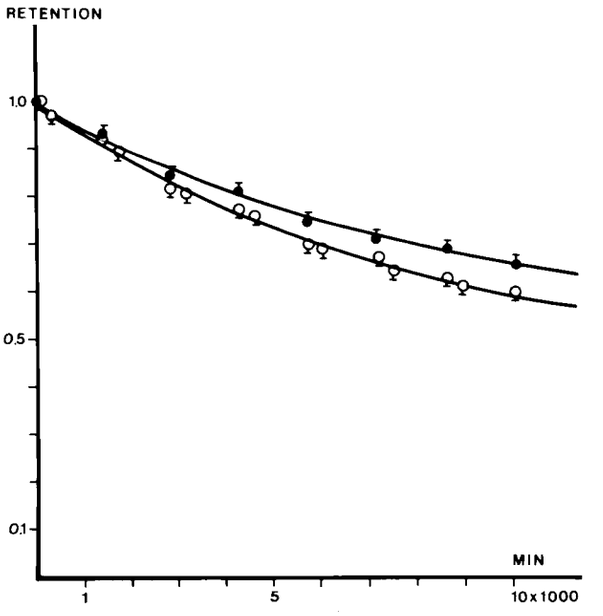

Fig. 2. Retention measurements in a normal individual showing the constantly increasing difference between the two retention curves. The upper ($R_2(t)$) calculated from urine and faecal excretion of ^{47}Ca activity, the lower ($R_1(t)$) directly measured by whole-body counting. Error bars (SD) are shown.

A_1 is calculated from A, b and λ, m, (u+d) and Δt were estimated from curve-fitting on WB retention data.

From the experimental data, two retention curves were calculated: $R_1(t)$ based on directly measured values, and $R_2(t)$ constructed from urinary and faecal excretion data. The two retention curves were different, $R_2(t)$ always being on a higher level than $R_1(t)$ with a constantly increasing difference between the curves during the investigation period as shown for a normal person in Fig. 2. The difference between the curves is regarded as caused by a cumulative dermal loss of activity estimated according to

$$R_2(t)-R_1(t) = d\times\int_0^t S^*(t)\,dt \tag{6}$$

where d = dermal calcium excretion rate.

Endogenous faecal calcium excretion rate (e) was calculated from

$$F^*(t) = e\times\int_0^{t-\Delta t} S^*(t)\,dt \tag{7}$$

where F*(t) is cumulated faecal activity.

Parameter estimation. Parameter estimation was performed by the principle of least squares. An effective iterative technique was needed because of non-linearity of the mathematical model (13).

Assessment of results. Variances were estimated according to

$$S^2 = 1/2n\sum_{i=1}^{n} d_i^2 \tag{8}$$

where n is the number of double determinations and d_i the difference between double determinations number i. The coefficient of variation was calculated as

$$CV\% = S\times 100/\bar{x} \tag{9}$$

where $\bar{x}$ is the mean value.

Whole-body (WB) counter. The applied WB counter was constructed as a stationary system of four crystals placed in an row beneath the stretcher. Each crystal was situated in an individual but fixed position to the body during measurements (15). The crystals were surrounded by lead cylinders with 7 cm thick walls and could be pushed in or out of the cylinders to obtain a higher or lower degree of efficiency and shielding.

Signals from the crystal-photomultiplier system were guided in parallel to a spectrometer followed by a scaler timer.

The effective detector to body distance ($\varrho(x,z)$ for the whole detector system was assessed to a first approximation (12) from the response of the detector system to a point source (x, z) placed in different positions in the horizontal plane through the body centre axis (Fig. 3) according to the equation:

$$\frac{1}{\varrho(x,z)^2} = \frac{1}{4}\sum_{i=1}^{4}\frac{EF_i}{(x-d_i)^2+z^2+D_i^2} \tag{10}$$

where EF_i is the relative crystal efficiency, d_i the horizontal distance from crystal to reference point (0.0), and D_i the vertical distance from crystal to stretcher (Fig. 3).

The geometric characteristic G(x, z) was then given by:

$$G(x,z) = \frac{\dfrac{1}{\varrho(x,z)^2}}{\dfrac{1}{\varrho(0,0)^2}} = \frac{\displaystyle\sum_{i=1}^{4}\frac{EF_i}{(x-d_i)^2+z^2+D_i^2}}{\displaystyle\sum_{i=1}^{4}\frac{EF_i}{d_i^2+D_i^2}} \tag{11}$$

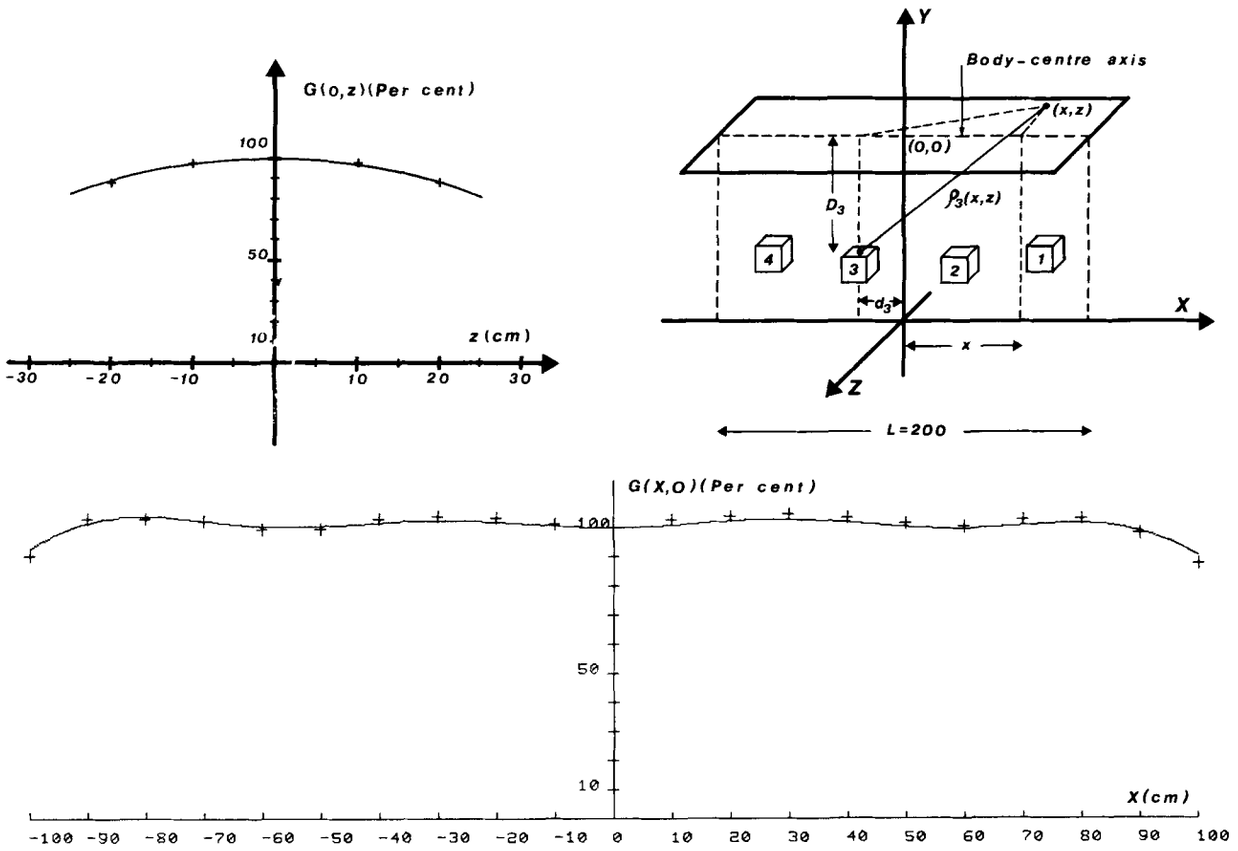

Fig. 3. Geometric characteristic of four-crystal stretcher system. Upper left: transverse response G(0, z) (+left, −right). Upper right: assessment of counter geometry for sources (x, z) in the centre plane through the body of a subject. ϱ_3 (x, z) is the effective detector to body distance, d_3 the horizontal distance from detector to reference point (0. 0) and D_3 the vertical distance from detector to the centre plane. Lower: longitudinal response G(x, 0) (+head, −foot).

The signal count-rate (SCR) (12) was calculated according to:

$$SCR = P \times \Omega \times \varepsilon_p \times F\,(cpm/kBq) \qquad (12)$$

where P is the photon emission rate of the body, Ω is the fraction of the photons emitted from the body which strikes the crystals, ε_p is the intrinsic photopeak efficiency and F is the fraction of counts in the photopeak that is recorded in a preselected energy interval.

Detection limit δ (12) was calculated by

$$\delta = 3 \times SD_{bg}/SCR\,(kBq) \qquad (13)$$

where SD_{bg} = standard deviation of local background radiation.

The subjects were counted shortly (one hour, no excretion) after the given dose. At the same time a reference source was counted. Each subsequent count of subject and reference source was compared with initial results to calculate the portion of dose retained.

Retention was calculated as

$$R(t) = \frac{\text{subject}(t) \times \text{reference}(0)}{\text{subject}(0) \times \text{reference}(t)} \qquad (14)$$

where all measurements were background corrected.

With this approach the subject was used as his own dose standard, which is valid as long as the distribution remains uncritical. The reference source was used to correct for physical decay and for variations in calibration and sensitivity of the instrument that may occur from day to day.

Dose estimation. The dose absorbed to different organs was estimated from MIRD formulation (7, 19). The assumption of radioactive equilibration between ^{47}Ca and its daughter isotope ^{47}Sc was used in the calculation. A mean time lag of two days in the bowel for ^{51}Cr was used in the calculations.

Results

Fig. 4 gives the *main parameters* for calcium metabolism (mean ± SE) obtained in 15 normal individuals. The analytical variation (coefficient of variation) for the individual parameters are given in parentheses.

The geometric characteristic for the WB counter

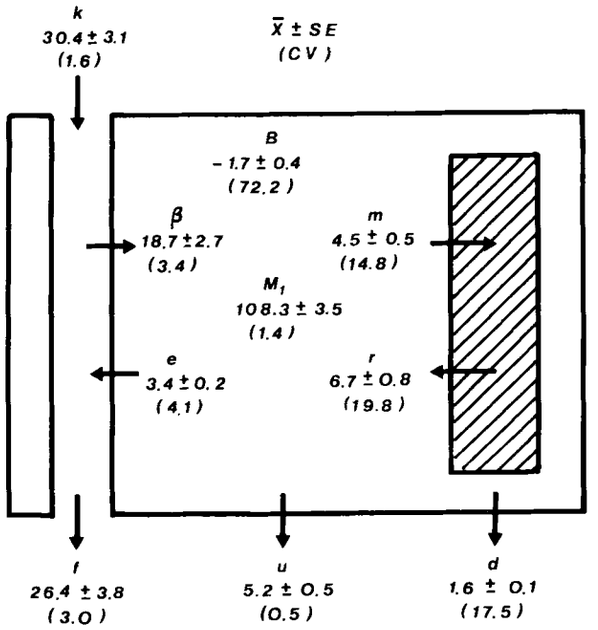

Fig. 4. Main parameters for calcium metabolism. Values are given as mean ± SE of 15 normal individuals. In parentheses method error in per cent of mean. All values are in (mmol/day) apart from M_1 (mmol) and β (per cent). Hatched area indicates mineralized bone. For the explanation of symbols, see p. 128.

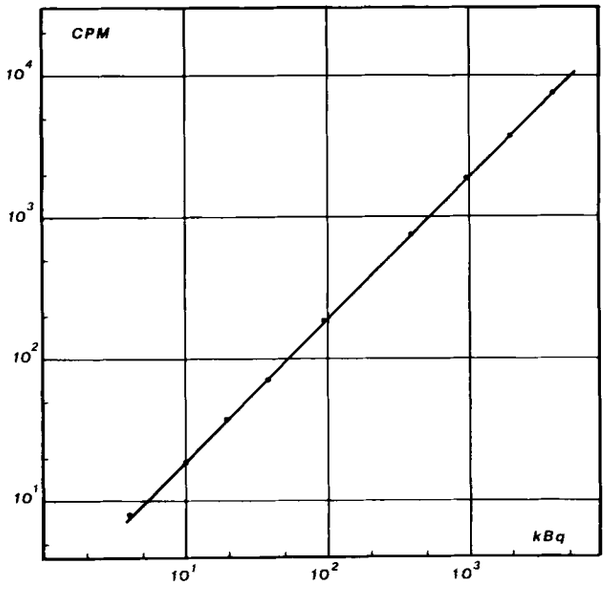

Fig. 5. Count rates versus phantom activity of a four-crystal stretcher WB counter system. cpm = 19×kBq+26, r=1.00, p<0.001, standard error of estimate (SEE)=58 cpm, n=9.

in transverse (G(0, z)) and longitudinal (G(x, 0)) direction is shown in Fig. 3. A close connection was found between the measured and calculated values. The average difference between the values was

Table 1

Reproducibility evaluated by calculation of method error and coefficient of variation

Retention	Mean	Error of method	Coefficient of variation (%)
In vivo (n=165)	0.8035	0.0054	0.67
In vitro (n=9)	0.2908	0.0029	1.00

0.59±0.31 (mean ± SE) per cent and did not differ significantly from zero (p>0.05).

It appears from the transverse curve in Fig. 3 that the geometric characteristic (G(0, z)) 10 and 20 cm from the body centre axis was 2.5 and 9.1 per cent, respectively, lower than the centre axis curve and nearly had the same shapes.

The linearity of the WB counter was evaluated by determining the net count rate versus phantom activity. Accurate samples of increasing activities of ^{47}Ca were diluted to 2 l in bottles with equal geometry and counted at the centre of the stretcher (Fig. 5). A high linearity was found (r=1.00, p<0.001, SEE=58 cpm) in the range 3.7 kBq to 3.7 MBq.

The level and variability of local background radiation (counting time 5.5 min) was estimated from 60 measurements over a period of 40 days to 813 cpm (mean) ± 30 cpm(SD) and with a CV=3.7 per cent.

The sensitivity in vivo was estimated according to formula (12) and found to be approximately

$$SCR \sim 18 \text{ cpm/kBq}$$

which gives a detection limit δ (formula 13) of about

$$\delta \sim 5 \text{ kBq } (0.14 \mu\text{Ci})$$

The reproducibility of the WB counter (Table 1) was estimated by 165 double determinations in 11 normal individuals and by 9 double determinations of point sources in the range 0.1 MBq to 1.0 MBq.

The dose estimation has been calculated by use of the model and the results obtained for normal individuals (Table 2).

Discussion

The described continuously expanding calcium pool model (4) has been used in the present investigation as it complies with modern concepts of bone remodelling and serum calcium homeostasis (17, 20). Moreover, the values obtained for mineraliza-

Table 2

MIRD dose estimation

Organ	Dose (mGy)
Total body	1.06*
Total bone	4.48*
Red marrow	3.42*
Ovaries	0.78*
Testes	0.68*
Lower large intestine (wall)	0.15**

* 0.74 MBq ^{47}Ca.
** 0.037 MBq ^{51}Cr/day for 10 days.

tion rate are independent of the part of serum specific activity/time curve chosen for analysis (10).

Whole-body counting was, as previously stated, regarded as an essential part of the ^{47}Ca turnover investigation, because the estimation of mineralization rate by this approach is not influenced by insufficient collections of faeces and urine or by variations in dermal calcium losses. If dermal calcium loss has to be determined, combined whole-body counting and collection of excreta are necessary (13).

When a radionuclide is administered for diagnostic purposes, the choice must be made either to give a relatively large dose which can be detected with a moderately sensitive counter or a smaller dose which demands an extremely expensive device with large detectors and heavy shielding. Dose estimation in this investigation showed a not negligible dose to especially red bone marrow. It was therefore desirable to reduce the amount of injected activity. This was possible even with the applied intermediate sensitive whole-body counter, because activity retained after 7 days (background radiation subtracted) was 74 to 111 kBq allowing a reduction of activity injected to a minimum of 0.37 MBq. The need for measurements of activity in excreta and especially serum makes this activity (0.74 MBq) necessary.

In general, it is not necessary for medical isotope diagnosis to determine the total radiation activity of the body in absolute units, relative values of successive measurements are sufficient, but a very good linearity is requested.

Changes in tracer distribution resulting in counting errors often make the interpretation of whole-body counting data difficult. The present detector system was optimized by help of a computer program to minimize the effects of the counter response from changes in body position and redistribution of radiation activity. It is, however, important to place the patient in almost the same centre position, longitudinally as well as transversely from measurement to measurement, as shown from the geometric characteristics. Quantitation of radionuclides, where the distribution changes with time, can be achieved in different ways (1). We have used phantom measurements and computer analysis to simulate ^{47}Ca going into deeper parts of the body (5). The phantom results have indicated that activity under 'worst case conditions' (i.e. 40 per cent of the activity in a small central part of the phantom) may 'disappear' with up to 2 per cent due to redistribution phenomena. This 'worst case redistribution' would result in a reduction in the calculated dermal calcium loss by 35 per cent.

Often, such as in measurements of radioactive iodine contamination, it would be satisfactory to obtain a WB counter reproducibility of 5 per cent (12). In relation to the often large variances obtained by computer estimation of derived parameters in physiologic studies, it is, however, important that the reproducibility is excellent, as found in our experiment (CV=0.7%).

The minimum detectable activity was derived from the following consideration: if the total count rate is different from the background count rate by more than 3 times the standard deviation of the background count rate, an effect has been observed with a probability of 99.7 per cent. The minimum detectable activity can therefore be considered to be the amount of activity resulting in a total count rate, that differs from the background count rate by 3 times the standard deviation.

As the background count is assumed not to be Poisson distributed, it is important to do long-time estimation of the background variation and to use this value in determining the detection limit.

The optimum advantage of whole-body counting in vivo must involve additional measurements (such as serum specific activity measurements), use of mathematical models and advanced computer calculations. In the present investigation, collections and analysis of stools and urine were necessary to gain information on endogenous faecal calcium excretion rate, dermal calcium excretion rate and calcium balance. Concerning bone turnover, only little information can be obtained from the whole-body gross values alone.

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