Elimination rate of $^{65}$Zn as a measure of food intake: a validation study in the mouse (Mus sp.)

J. A. McLEAN, AND J. R. SPEAKMAN
Department of Zoology, University of Aberdeen, Aberdeen AB9 2TN, Scotland, United Kingdom

McLean, J. A., and J. R. Speakman. Elimination rate of $^{65}$Zn as a measure of food intake: a validation study in the mouse (Mus sp.). J. Appl. Physiol. 79(4): 1381–1389, 1995.—We measured elimination of $^{65}$Zn in white mice (Mus musculus) using daily whole body counting. Thirteen male mice were randomly divided into three groups, each maintained at a different temperature. Each animal was labeled with $^{65}$Zn on day 0 and monitored over days 0–48 postinjection. Daily food intake and body masses of all the animals were measured. We evaluated the ability of derived components of the $^{65}$Zn elimination curves to predict food intake over different phases of the measurement period. Food intake was significantly different between temperature groups; temporal variation in food intake was not intercorrelated between groups. Whole body elimination of $^{65}$Zn involved a rapid decline over days 0–1, followed by a biphasic decline in counts over days 1–48. Components of the first phase of the biphasic elimination curve were not significantly related to food intake. The rate ($k_2$) of isotope elimination in the second phase was significantly related to mean food intake over days 25–48, 13–24, and 37–48. Rate of turnover in the second phase of elimination, incorporating the variation in zinc body pool size ($b_2 = 1/N_2$), where $N_2$ is the constant of the second phase of elimination, was the best predictor of food intake and accounted for 60% of the variability over days 37–48.

ENERGY ALLOCATION STUDIES are often hindered by the absence of a simple and reliable means of measuring food intake (3, 10). The conventional method of isolating animals in metabolic cages to monitor individual food intake is labor intensive, and the disruption of normal social interaction by isolation may be undesirable in relation to other aspects of investigation. For example, in some studies of the role of dominance in nutrition, it is necessary to know the share of a communal food source that is consumed by each of several animals housed together. An alternative approach to this problem is to use elimination rates of tracer injections of radioactive isotopes. The majority of studies in which radioisotope techniques are used for estimating food intake have ultimately also attempted to use the turnover as a measure of metabolic rates (18). This assumes that animals eat no more or less food than they require to satisfy their metabolic requirements (i.e., the animal is in energy balance and hence metabolism is directly related to, and accounts for, the largest portion of food energy consumption). Early attempts to correlate elimination rates of radioisotopes with metabolic rates were rather inaccurate, e.g., $^{137}$Cs and $^{32}$P (2), $^{65}$Zn and $^{54}$Mn (4), and $^{131}$I (14). Radiosodium ($^{22}$Na) elimination has received recent attention as a measure of food intake in a number of animals: dingoes Canis familiaris (10); captive rabbits Oryctolagus cuniculus (12); lions Panthera leo and spotted hyenas; Crocuta crocuta (11), and penguins Eudyptula minor and Pygoscelis papua (7, 8). However, $^{22}$Na has a relatively short biological half-life, which may be insufficient to obtain a representative estimate of average food intake. For example, in 1-kg penguins, the half-life for $^{22}$Na was 3 days (7). Moreover, some techniques for measuring turnover of $^{22}$Na require blood sampling and the subsequent preparation necessary for scintillation counting. Finally, some animals may turn over sodium because of intakes of this element that are unrelated to food intake. For example, marine animals may have significant intake of sodium from seawater.

It has been long established that the elimination of an injection of radiolabeled zinc $^{65}$Zn by mammals is related to food intake (4) and, specifically, to levels of dietary zinc intake (5, 19). However, early studies in which whole body elimination rates of $^{65}$Zn were used were not sufficiently sensitive to serve as good measures of individual food-intake rates (4, 15). Despite the equivocal nature of these early studies, it has not been conclusively shown that the use of $^{65}$Zn is without value as a measure of food intake. Furthermore, the use of this radioisotope has several advantages over other isotopes, for example, because of the technical simplicity of the method (whole body counting). Second, $^{65}$Zn has a relatively specific pathway through the body and is excreted mostly in the feces (5, 17, 19). Third, $^{65}$Zn has a suitable biological half-life (e.g., 18.9 days for the third phase of elimination in Peromyscus polionotus) (4) and a physical half-life (244 days) to enable application of the method in the field. A longer biological half time has advantages, because the resultant measure is less prone to error due to the stress of capture. Measurements over longer periods even out day-to-day variation in food intake to give a representative average. Furthermore, animals are more likely to be in long-term energy balance (if evaluation of metabolism is desirable), as mass changes will contribute little to the total energy budget over a longer period. Finally, there are few exogenous sources of zinc available to animals apart from food. Advances in counting technology over the two decades since the early studies were made may now present the possibility of using $^{65}$Zn as a method to measure individual food energy-intake rates. In this paper, we evaluated the use of $^{65}$Zn to measure food intake in the laboratory mouse when feeding on a uniform diet. This represents the best-case scenario for validating the technique, as variations in dietary zinc content were minimized.

MATERIALS AND METHODS
All experiments described in this paper were performed under project and personal license authority of the UK Home Office.
Animals and housing. A total of 13 male white mice (MF1, aged 40 days at the time of labeling) were used in the experiments. Mice were randomly allocated to one of three groups and housed in controlled-temperature incubators (Gallen-camp; INL-401N-010) in which they were subjected to a 10:14-h light-dark photoperiod (lights on at 0800). Each group of mice was maintained at a different constant temperature: group 1, three individuals at 14°C; group 2, five individuals at 21°C; and group 3, five individuals at 27°C (previous studies have indicated lower critical temperature = 28–30°C). Mice were allowed 10 days to become adjusted to the housing and temperature treatments before they were labeled. During the preliminary and the experimental periods, mice were housed individually in plastic "shoebox" cages (30 × 12 × 12 cm) with stainless steel wire lids and given free access to water and food provided ad libitum (rat and mouse breeding diet, SDS, Witham, UK). All the food used throughout the experiment came from a single batch for which the manufacturers estimated zinc content was 61 mg/kg. A layer of sawdust was provided on the floor of each cage for bedding purposes. Food intake for individual mice was measured daily by subtracting the mass of uneaten food pellets in the hopper from the mass of food supplied. Mice were weighed daily, immediately before counting. All food and body mass measurements were made using a top-pan balance with a precision of 0.01 g (Sartorius).

Water was freely available to the animals throughout the experiments. Local tap water has a mean zinc content of ~40 μg/l (Regional Water Services data). If the animals drank the same mass of water as they ate food each day, the contribution of water zinc to their total zinc intake would be <1/1000th that of the food intake.

Administration of radioisotope and counting procedure. The zinc isotope (100 mCi/mmol : 3.7 Bq/mmol) was in the chloride form dissolved in 0.086 M HCl, which was diluted with water to obtain a concentration of 10 μCi (370 kBq)/ml. Each mouse was labeled with 65Zn in a single intraperitoneal 0.15-ml injection containing 1.5 μCi (55.5 kBq). The day of injection was defined as day 0 of the experimental period. Whole body counts were taken within 3 h postinjection and then almost daily for 43 out of 48 days by using a detector crystal, at a distance of 20 em from the surface of the detector crystal. Physical decay has been ignored in several previous studies (e.g., Refs. 17, 19) but not in others (e.g., Refs. 5, 13, 23). These previous studies have suggested that the decision whether to ignore physical decay appears to have no influence on the accuracy of the technique. We therefore made calculations both disregarding and accounting for the physical decay of 65Zn to assess the effect of this procedure.

Distribution of 65Zn in tissues. A total of six individuals (two from each treatment group) were dissected at the end of the counting period (50 days postinjection). Carcasses were divided into 12 tissue/organ groups: liver, spleen, kidney, heart, lung, thyroid, fat, reproductive organs, gut, skin, muscle, and skeleton. Organs and tissues were weighed and counted for radioactivity (count time = 600 s). Items to be counted were placed between 2 watchglasses and fixed centrally, directly against the surface of the detector. Counts per 600 s were corrected for background by subtracting counts obtained by placing two empty watchglasses in front of the detector crystal. The distribution of 65Zn present in organs and tissues was expressed as mean counts per gram of wet tissue across the six carcasses.

Statistics. Patterns of food intake in relation to temperature and time were analyzed using two-way analysis of variance (ANOVA) and least squares linear regression analysis. Exponential functions were fitted to the whole body elimination data by using an iterative nonlinear curve-fitting program (BMDP Statistical Software, 1990, program 3R). Relationships between rate of isotope elimination and food intake were investigated using linear regression analysis with parameters from the iteratively fitted curves as the dependent variables. The distribution of radioisotope present in organs and tissues was examined using one-way ANOVA.

RESULTS

Food intake. Mean food intakes (calculated for 6-day intervals over days 1–48) for the three temperature groups (14°C group 1; 21°C group 2; 27°C group 3) involving a total of 13 individual mice are shown in Fig. 1. Food intake was significantly different between temperature groups (two-way ANOVA, F = 485, df = 2,170, P = 0.0001). Mean food intake (g/day) over days 1–48, averaged across all individuals and days, for groups 1, 2, and 3 was 8.76 ± 0.09 (SE) g/day, n = 123; 6.92 ± 0.07 (SE) g/day, n = 205; and 4.73 ± 0.09 (SE) g/day, n = 205, respectively. Food intake was significantly variable over time for groups 1, 2, and 3 (ANOVA, F = 3.26, df = 7,161, P < 0.01). The variation in food intake for each group was not directional: least squares regression analysis (calculated using individual data from each temperature group) indicated no significant relationship of food intake with time for any group. Hence, there was a significant difference in food intake.
between groups, and within these groups there was a
non-directional variation in food intake with time. The
non-directional variation was not intercorrelated be­
tween groups (ANOVA group × time interaction, \( F =
4.24, \text{df} = 14.163, P < 0.0001 \)).

Whole body elimination of \( \text{^{65}Zn} \). A typical plot of
whole body counts in relation to time over \( \text{days 0–48} \)
is illustrated in Fig. 2. Visual inspection of the elimina­
tion curves revealed a rapid decline in counts over days
\( \text{0–1} \), followed by a curvilinear decline in whole body
counts through to day 48. The initial postinjection
count (taken within 3 h after injection) was excluded in
the analysis for each elimination curve. The remaining
data (i.e., \( \text{days 1–48} \)) were fitted to a biexponential
model of the form

\[
y = N_1 e^{k_1 t} + N_2 e^{k_2 t}
\]

where \( y \) is whole body counts per 120 s corrected for
background variation; \( t \) is time (days postinjection); \( k_1 \)
and \( k_2 \) are biologic elimination constants (or nonlinear
gradients of decline) for phase 1 and phase 2 of elimina­
tion; and \( N_1 \) and \( N_2 \) are intercepts for phase 1 and 2
of elimination. The values for \( N_1, k_1, N_2, \) and \( k_2 \)
were calculated by an iterative curve-fitting program
(BMDP, 3R). The biologic elimination constants \( k_i \) and
\( k_j \) described the rate of decline in whole body counts
and reflected the rate of elimination of \( \text{^{65}Zn} \) from
each individual. The intercepts \( (N_1 \) and \( N_2 \)) of individual
isotope-elimination curves reflected (inversely) the size
of body pools of zinc.

The biexponential function for individual elimination
curves was back extrapolated to calculate a predicted
number of counts for day 0. The observed counts on
day 0 were significantly higher than the level of counts
predicted from the model (paired \( t \)-test, one tailed:
mean = 21,427 ± 671 \( \text{(SE)} \), \( SD = 2,418, t = 31.96, P <
0.0001, n = 13 \)). This confirmed our visual interpreta­
tion that the day 0 count did not lie on the elimination
curve, calculated for the remaining data.

Typical elimination curves for \( \text{^{65}Zn} \) (excluding counts
on day 0) and fitted biexponential functions for three
mice (one individual from each temperature group) are
shown in Fig. 3. All of the individual elimination curves
were characterized by an initial phase of rapid decline
in counts \( (k_1 = -0.133 \text{ to } -0.664) \) followed by a second
phase of loss at a slower rate \( (k_2 = -0.024 \text{ to } -0.039) \).
Values for the elimination constants \( (k_1 \) and \( k_2 \)) and
intercepts \( (N_1 \) and \( N_2 \)) of the fitted curves for all the
individual animals are presented in Table 1.

Visual inspection of the individual elimination
curves (e.g., Fig. 3) revealed an apparent intercorrela­
tion across individuals of the daily deviations from the
overall elimination curve. Because food intake vari­
atations were not temporally intercorrelated (Fig. 1), this
apparent intercorrelation suggested that variation in
countercurrent efficiency might be contributing to error
in the daily whole body counts. We correlated the normalized
residuals to the fitted elimination curves across all poss­
able animal pairs and found that all these correlations
were significant (Table 2), indicating that day-to-day
variation in countercurrent efficiency was important.
Because we did not count a daily standard, we could not
retrospectively correct the data for this day-to-day variation
in countercurrent efficiency. However, since all the animals
curves of radiolabeled zinc for
have had an equivalent effect on all the elimination
were counted simultaneously over the same time pe­
riod, any systematic bias in counterefficiency would
have had an equivalent effect on all the elimination
curves across all the individual animals. Thus these
errors would be unlikely to affect the significance of
any relationship between isotope elimination and food
intake.

Rate of isotope elimination in relation to food intake.
The biexponential functions fitted to the individual iso­
etime intake over different stages of the experimental
time periods, any systematic bias in counterefficiency would
errors would be unlikely to affect the significance of
any relationship between isotope elimination and food
intake.

<table>
<thead>
<tr>
<th>Table 1. Elimination constants (k1 and k2) and the intercepts (N1 and N2) of both phases of elimination curves of radiolabeled zinc for 13 individual mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
</tbody>
</table>

TABLE 2. Correlation coefficients (r) of daily normalized residuals to elimination curves of radiolabeled zinc across all possible pairwise comparisons of 13 individual mice over 48 days after labeling

<table>
<thead>
<tr>
<th>Mouse</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.676</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.571</td>
<td>0.805</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.652</td>
<td>0.889</td>
<td>0.825</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.472</td>
<td>0.758</td>
<td>0.740</td>
<td>0.782</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.518</td>
<td>0.793</td>
<td>0.757</td>
<td>0.814</td>
<td>0.802</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.579</td>
<td>0.573</td>
<td>0.593</td>
<td>0.574</td>
<td>0.532</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0.723</td>
<td>0.854</td>
<td>0.785</td>
<td>0.861</td>
<td>0.756</td>
<td>0.805</td>
<td>0.634</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0.359</td>
<td>0.645</td>
<td>0.547</td>
<td>0.678</td>
<td>0.673</td>
<td>0.558</td>
<td>0.502</td>
<td>0.665</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.500</td>
<td>0.766</td>
<td>0.814</td>
<td>0.832</td>
<td>0.753</td>
<td>0.737</td>
<td>0.611</td>
<td>0.796</td>
<td>0.734</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>0.497</td>
<td>0.759</td>
<td>0.649</td>
<td>0.758</td>
<td>0.761</td>
<td>0.663</td>
<td>0.457</td>
<td>0.752</td>
<td>0.791</td>
<td>0.757</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>0.703</td>
<td>0.767</td>
<td>0.793</td>
<td>0.769</td>
<td>0.688</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>0.652</td>
<td>0.733</td>
<td>0.759</td>
<td>0.744</td>
<td>0.591</td>
<td>0.707</td>
<td>0.632</td>
<td>0.787</td>
<td>0.368</td>
<td>0.770</td>
<td>0.802</td>
<td></td>
</tr>
</tbody>
</table>

All r values were significant; P < 0.01.

Regression analysis was performed for phase 1 and 2 of biexponential decline in whole body counts of 65Zn for 13 individual mice after single injection of label. Mean food intake was calculated over various time categories of the measurement period. * P < 0.05; ** P < 0.01; *** P < 0.001 (Table 3). Regressions of k1 and mean food intake calculated over all other time categories resulted in borderline significance (P = 0.053 to P = 0.073), except for days 1-12 (P = 0.176) (Table 3). In addition, we investigated the effect of incorporating body pool size (reciprocal of intercepts for phases 1 and 2) in the regressions of elimination rate of isotope and mean food intake. This involved a similar set of regressions using the same time categories for the calculation of food intake but using k1(1/N1) and k2(1/N2) as the dependent variables. There

FIG 1. Elimination of 65Zn as measure of food intake in mice.
The mean mass of experimental animals (averaged us 
\( \frac{\text{ing h2}}{\text{alone (Table ;»)}} \) The variable 

\[ \text{cross all groups} \] increased from 28.04 
\[ \text{and mean food intake for individual mice calculated} \]
\[ \text{x} \]
\[ \text{37--48 after a single injection oflabeD in relation to} \]
\[ \text{se} \]
\[ \text{3 individual mice, Least squares fit regression equation is illus­} \]
\[ \text{trations of} \]
\[ \text{urately predicted mean food intake in individual} \]
\[ \text{the significance of any of the relationships.} \]
\[ \text{ke (giday) allow investigation of tissue distribution of} \]
\[ \text{up} \]
\[ \text{en} \]
\[ \text{issue distribution or} \]
\[ \text{ed by fitted line: gradient} \]
\[ \text{x intercept} = -1.14 \]
\[ \text{1/intercept = -1.14} \]
\[ \text{32 food} \]
\[ \text{during whole body counts of} \]
\[ \text{regressions in­} \]
\[ \text{were significant for all time categories, and levels} \]
\[ \text{ne were significant for all} \]
\[ \text{d experimental period ranged from 2G,000 to 6G,000.} \]
\[ \text{e were significant for all time} \]
\[ \text{e was no significant relationship between} \]
\[ \text{k}_{2} (1/N_{2}) (\text{phase} \]
\[ \text{and mean food intake for individual mice calculated} \]
\[ \text{over any of the time intervals} \]
\[ \text{P > 0.05 all cases, Table} \]
\[ \text{The regressions of} \]
\[ \text{most accurately predicted mean food intake in individual} \]
\[ \text{over days 37-48; the least squares fit regression} \]
\[ \text{over this interval (Fig. 4).} \]
\[ \text{The mean mass of experimental animals (averaged} \]
\[ \text{across all groups) increased from 28.04 ± 0.54 (SE) g} \]
\[ \text{on the day of injection to 35.30 ± 0.78 g on day} \]
\[ \text{postinjection. There was no significant relationship} \]
\[ \text{between} \]
\[ \text{and mean mass change} \]
\[ \text{we recalculated all the elimination con­} \]
\[ \text{cons} \]
\[ \text{tants after correcting the original data for the anticip­} \]
\[ \text{ated physical decay of the isotope. This had no effect} \]
\[ \text{on the significance of any of the relationships.} \]
\[ \text{Tissue distribution of} \]
\[ \text{Whole body counts (per} \]
\[ \text{for the examined carcasses (n = 6) at the end of} \]
\[ \text{the experimental period ranged from 26,000 to 66,000.} \]
\[ \text{To allow investigation of tissue distribution of} \]
\[ \text{using all carcass data, counts obtained for each tissue} \]
\[ \text{group were initially expressed as a percentage of the} \]
\[ \text{whole} \]
\[ \text{mean} \]
\[ \text{counts} \]
\[ \text{were recalculated for individual carcasses} \]
\[ \text{assessing a standard body count of 50,000. One­} \]
\[ \text{way ANOVA revealed a marginally significant effect} \]
\[ \text{of tissue/organ group on the distribution of} \]
\[ \text{one-way ANOVA:} \]
\[ \text{although all possible pair combinations of mean counts} \]
\[ \text{per gram wet tissue were not significantly different (Tukey} \]
\[ \text{method for minimum significant difference} \]
\[ \text{(20). The variance and thus the ANOVA were heavily} \]
\[ \text{influenced by a single very high spleen count. When} \]
\[ \text{this data item was excluded from the analysis, the} \]
\[ \text{significance of the organ/tissue group effect on the distri­} \]
\[ \text{bution of} \]
\[ \text{was greater} \]
\[ \text{ANOVA:} \]
\[ \text{P < 0.01}. \]
\[ \text{Pairwise comparisons of the adjusted} \]
\[ \text{Tukey} \]
\[ \text{revealed that the mean counts per gram} \]
\[ \text{tissue were significantly higher in the thyroid than} \]
\[ \text{in fat, gut, muscle, kidney, spleen, or liver. There was} \]
\[ \text{was a high level of counts in the} \]
\[ \text{although this was not significantly different from any} \]
\[ \text{other tissue} \]
\[ \text{P > 0.05}. \]

**DISCUSSION**

Whole body counts of \(^{65}\)Zn after a single injection of \(^{15}\) label displayed a rapid decline in counts over the first day postinjection. We excluded this initial decline in our analysis. We eliminated the initial postinjection count (day 0) on the assumption that the injected \(^{65}\)Zn had not yet integrated with the body pool. It is unlikely that the \(^{65}\)Zn had been integrated with the body pool within the 3-h period between injection and counting on day 0 for animals of this size (25-35 g), since even \(^{18}\)O and \(^{22}\)Na (examples of isotopes with rapid turnover rates) have equilibration times of ~1.5 h (see Ref. 21, *Pelecatus auritus, 7–10 g*) and 6 h (see Ref. 7, *Eudyptula minor, 1 kg*), respectively. The finding that the day 0 count did not lie on the elimination curve, calculated by backextrapolation of the biexponential for the remaining data, provided further supportive evidence for our assumption that during this early phase the isotope would not have pervaded the body pools yet. Whole body counts from day 1 onward followed a biexponential decline, consisting of a first phase of rapid elimination followed by a second phase of elimination at a slower rate. The pattern of decline in whole body counts of \(^{65}\)Zn described in previous studies appears to depend on several factors. These include the length of the experimental period and the timing of the first postinjection count to be included in the elimination curve. Chew (4) reported a three-component exponential de-
cline in whole body counts after injection of $^{65}$Zn, when using an initial count taken 8 h postinjection and a measurement period of 38 days. Liu-Sheng et al. (13) also detected a three-phase exponential decline in counts of $^{65}$Zn, but their analysis used a starting count taken on the first day after injection and a maximum experimental period of 100 days for adult mice. In comparison, orally administered radioisotope produced a two-phase elimination pattern (9). This latter study (9) involved a starting count taken immediately postinjection and an experimental period of 30 days. In the present study, the measurement period was 48 days, and we detected a two-phase pattern of elimination starting with counts taken on day 1 postinjection and a three-phase pattern if the rapid elimination over the first day was included.

The mechanism underlying the elimination process is not fully understood, although it has been demonstrated that zinc metabolism is controlled by at least two homeostatic mechanisms that act at the sites of absorption and of intestinal excretion (6). These homeostatic mechanisms would act to produce elimination rates of $^{65}$Zn related to levels of dietary zinc intake, as found previously (3, 19). This also explains the relationship between rate of elimination and food intake found in the present study. However, such a simple mechanism would produce a monoexponential decline, and the situation is clearly much more complex than this. When the data are pooled across all the previous studies in combination with the present study, there are probably four phases of isotope elimination detectable using whole body counting. A diagrammatic representation of these phases of the elimination process is given in Fig. 5.

**Phase 1.** After injection of label, some $^{65}$Zn appears to be rapidly eliminated, probably in the feces and before any integration with the body pool. This phase is reflected in the very rapid initial decline in counts over the first 24 h postinjection.

**Phase 2.** $^{65}$Zn integrates with an exchangeable body pool and expands the size of the body pool. $^{65}$Zn is then rapidly eliminated during the equilibration process to reestablish the size of the body pool. **Phase 2** is equivalent to the first phase of the biexponential decline in the present study ($k_1/N_1$). Because the pool is reequilibrating, the elimination need bore no relation to the flux of zinc through the entire pool. This accords with the absence of any significant relationship between food intake and the parameters of the first phase of the elimination curve described in the current study.

**Phase 3.** $^{65}$Zn equilibrates with zinc coming into the system from food and is eliminated at an intermediate rate along with stable zinc in the feces. **Phase 3** is equivalent to the second phase of the biexponential decline in the present study ($k_2/N_2$). Because in this phase the pool size is stable, the elimination rate is closely linked to dietary zinc intake. This accords with the significant relationship between parameters of the elimination curve and food intake for this phase found in the current study. During this phase, there may also be losses from the free body pool occurring to body components, but the magnitude of loss from these pools is small relative to that in the feces during this phase. Chew (4) reported that **phase 3** of his elimination curves was the best predictor of food intake. It is likely that **phase 3** in Chew’s study was equivalent to the second part of the biexponential decline in the present study, since Chew used a starting count taken 8 h postinjection. The high predictability for food intake in **phase 3** in Chew’s study is in accord with the model developed here. In the present study, the ability to predict food intake was not only dependent on the gradient of decline but also on the reciprocal of the intercept, i.e., the size of the body pool. This would be expected given the model presented in Fig. 5, as elimination would be more rapid from a smaller pool. The effect of body pool size was not investigated by Chew. The explained variation for the relationship between cumulative food intake and the slope of decline in whole body counts of $^{65}$Zn found by Chew was 45%. By incorporating body pool size in our regressions, the predictability of the relationship between turnover of $^{65}$Zn for individual mice and mean food intake over days 37–48 was raised from 45% to 65%. This improvement in predictability in comparison to early studies for **phase 3** indicates that the use of $^{65}$Zn should not be overlooked as a method for measuring food intake. However, it should be borne in mind that on a uniform diet the current study represents a probable best case, and further studies of animals feeding on natural food with potentially more variable zinc contents are required.

**Phase 4.** If $^{65}$Zn is incorporated into slowly exchanging tissue pools, then, dependent on the rate of exchange of these tissue pools, there may be a fourth phase of slow elimination. We did not detect this phase in our study. The third phase of elimination reported by Liu-Sheng et al. (13) may be equivalent to this proposed fourth phase of elimination, since by starting with counts on day 1, they probably excluded our proposed **phase 1** of elimination, and by extending the measurement period to 100 days they may have detected an additional phase not reached in the present study. The presence of poorly exchangeable zinc pools was suggested by Rubini et al. (17) when they reported difficulty in displacing $^{65}$Zn in the body with nonradioactive zinc. Schryver et al. (19) documented greater percentages of $^{65}$Zn in liver, pancreas, kidney, heart, and lung than in structural organs such as bone and muscle (7 and 14 days after dosing). Rubini et al. (17) reported injected $^{65}$Zn chloride was first detected in soft tissues, and bone accretion followed much later. In the present study, mice were killed 50 days postinjection, and relatively high levels of $^{65}$Zn were present in the thyroid with a tendency for high levels (not significant) in the skeleton. Levels of $^{65}$Zn between all other body components were not significantly different. In view of these observations, it seems likely that $^{65}$Zn is lost to slowly exchangeable tissue pools, for example, the bone matrix. Individual differences in the movement of $^{65}$Zn back from slowly exchanging pools during the third phase of elimination may account for some of the discrepancy between food intake and turnover estimates. Variation in counterefficiency appeared to contribute to some of the observed variation in counts (Table 2).
**ELIMINATION OF $^{65}$Zn AS MEASURE OF FOOD INTAKE IN MICE**

Phase 1. Injection. Rapid loss of some isotope prior to equilibration, direct from injectate.

Phase 2. Rapid elimination of $^{65}$zinc during equilibration. Pool re-establishing size.

Phase 3. Intermediate rate of elimination of $^{65}$zinc dependent primarily on the flux of Zn in food.

Phase 4. Slow rate of elimination of $^{65}$zinc dependent on rate of exchange from poorly exchangeable tissue pools.

**Diagramatic model of proposed isotope-elimination process involving 4 phases of decline in whole body counts of $^{65}$Zn after a single injection of label.**
The use of a standard to correct readings for variation in counterefficiency would eliminate this problem and is advised for future studies. This would be of particular importance where different individuals were not counted over the same period of days, and thus systematic bias in counterefficiency would have different effects in the elimination curves of different individuals. In the present study, the absence of the use of a standard to correct the readings was unlikely to have contributed to the residual error in the correlation of turnover to food intake, since all the animals were studied over the same 48-day period.

Various other factors may also affect $^{65}$Zn elimination and should perhaps be considered as potential areas for control to improve the technique. Zeigler et al. (23), for example, found that elimination of $^{65}$Zn was lower in zinc-deficient animals than in zinc-sufficient animals. Robertson and Burns (16) reported that diets of low zinc and high calcium in dogs produced higher elimination rates of $^{65}$Zn in feces compared with controls. The effects of food intake on elimination rate in both these studies were not reported.

In conclusion, we have presented a model for the process of $^{65}$Zn elimination consisting of a four-phase exponential decline in whole body counts. Parameters of the isotope-elimination curve in phase 3 provided the best predictor of food intake (i.e., $\sim 35-50$ days postinjection). Incorporation of body pool size in relationships between the rate of isotope decline and food intake gave an improvement in predictability over early studies. Throughout the study we have assumed that animals are available for counting on a daily basis. This is not likely to present a problem in the laboratory; however, this criterion is unlikely to be met in the field where the number of multiple recaptures will be limited depending on the particular study species. Adaptation of the method for use in the field would require a complex protocol, since animals would need to be captured and injected with radiolabel and then subsequently recaptured at 35 and 50 days postinjection for whole body counting during the third phase of elimination. We evaluated the potential of the method to predict food intake where it was only possible to recapture animals twice after injection. Least squares regression analysis of the relationship between the gradient of isotope decline (calculated by using the log-converted values for individual counts on days 37 and 48) and mean food intake (calculated for individual mice over the same period) resulted in borderline significance (gradient = $-0.0131 - 0.00135$ mean food intake; $r^2 = 0.24$, $R = 3.40$, df = 1,11, $P = 0.092$). There was a significant relationship between (gradient $\times 1/\text{intercept}$) and food intake (calculated using the same protocol): (gradient $\times 1/\text{intercept}$) = $-0.00135 - 0.000171$ mean food intake ($r^2 = 0.32$, $R = 0.24$, df = 1,11, $P = 0.043$). Hence the explained variation in food intake was substantially reduced when a two-point predictor of food intake was implemented, although the outcome was still significant.

The use of $^{65}$Zn elimination as a method for measuring food intake has a number of potential applications. Within single populations of animals, individuals may vary considerably in energy utilization and the possession of a simple technique for comparing individual food intake rates is a valuable asset for any study of individual variation in energy allocation. The turnover of $^{65}$Zn may be a valuable technique for the measurement of food consumption in captivity, for example, where animals are kept in group situations and measures of individual food intake are required without isolation.

Implementation of the method in the field may be restricted to animals that have a high recapture rate (since the accuracy of the technique was reduced using a two-point predictor). There would also be logistic limitations on maximum body size of study animals capable of being counted by a crystal detector. Moreover, in the field, animals are unlikely to ingest food that has a constant and nutritionally adequate content of zinc. If dietary zinc sources were more impoverished and variable, this might also compromise use of the technique in the field. For example, if dietary zinc contents were low, then the elimination due to food passage through the animal might also be low, and at some point noise in the measurements might compromise definition of the elimination constant. Variation in dietary sources of zinc might also increase day-to-day variation in the extent of elimination. Over the long term, such effects might not be significant if the mean rate of zinc intake was high. However, increased variation in daily counts would compromise the definition of the elimination constant, and this would be most serious where the average level of dietary zinc was lower. In combination then, a supply of zinc that was both impoverished and variable would be likely to seriously affect the efficacy of the technique. The most serious situation compromising the technique would be where a systematic shift in the diet occurred between sources of differing zinc content.

These considerations suggest that the technique may not be of particular use in the field, and further validation work would be required to establish its usefulness in any specific circumstance. If these problems can be overcome, the turnover of $^{65}$Zn may be a useful alternative technique to, for example, the turnover of $^{22}$Na as a measure of food intake where estimates over protracted periods are required.

Address for reprint requests: J. A. McLean, Dept. of Zoology, Univ. of Aberdeen, Aberdeen AB9 2TN, Scotland, UK.

Received 15 June 1994; accepted in final form 2 May 1995.

REFERENCES


ELIMINATION OF 66Zn AS MEASURE OF FOOD INTAKE IN MICE


