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TURNOVER OF ^{65}Zn AND ^{85}Sr IN GROWING RATS

A comparative investigation

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The turnover of ^{65}Zn in the mammalian body has been the subject of many investigations using either whole-body autoradiography (BERGMAN & SÖREMARK 1968), microautoradiography (HAUMONT 1961, HAUMONT & MCLEAN 1966, JOWSEY & ORVIS 1967) or quantitative scintillation techniques (BALLOU & THOMPSON 1961, CZERNIAK et coll. 1962, STRAIN et coll. 1964, BERGMAN 1970, MCINTOSH & LUTWAK-MANN 1972). These investigations have shown ^{65}Zn to be rapidly taken up and lost by such viscera as kidney, pancreas, liver and spleen and by the gastrointestinal tract. It is moderately rapidly taken up by the skeleton and the activity remains in bone for a very long time. The rate of the uptake and loss in the central nervous system is slow and in skeletal and heart muscle it is intermediate.

In preparation for a comparison of the turnover of ^{65}Zn in rats fed a zinc-deficient diet with and without a zinc supplement, it was necessary to obtain more complete knowledge of how ^{65}Zn turnover reflects the turnover of zinc in various tissues and organs, particularly in bone. For this purpose the present attempt was made to analyze quantitatively the turnover of ^{65}Zn in the tissues of growing rats using the model of BAUER et coll. (1955 a). Two isotopes, ^{65}Zn and ^{85}Sr, were injected simultaneously into the same animals in order that direct comparisons could be made between the calculated parameters for zinc and those for calcium (^{85}Sr).

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Materials and Methods

Forty-eight female, albino rats of the Sprague-Dawley strain were used. The animals were raised in our laboratory from 3 weeks of age, just after weaning. They were housed in cages of acrylic resin with stainless steel covers. They had access to ordinary tap water and were fed a conventional pellet diet ad libitum (210 Anticimex, Anticimex, Stockholm).

At six weeks of age the rats were injected intraperitoneally with both ^{65}Zn and ^{85}Sr. The ^{65}Zn, as zinc chloride in 0.1 N HCl, had a specific activity of 118 mCi ^{65}Zn/mg zinc, and the ^{85}Sr, as strontium chloride in aqueous solution, had a specific activity of 9.2 mCi ^{85}Sr/mg strontium (the Radiochemical Centre, Amersham). The injection solution was prepared by mixing portions of the two stock solutions and diluting with physiologic saline to final concentrations of 40 μCi ^{65}Zn and 25 μCi ^{85}Sr per ml of injection solution. The injected doses were 0.2 μCi ^{65}Zn and 0.125 μCi ^{85}Sr per gram body weight.

Four rats, randomly selected, were killed at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16 and 24 days following the injection. The animals were lightly anaesthetized with ether and killed by decapitation. The sampling techniques and preparation of samples for the determinations have been described previously (BERGMAN 1970). Samples of serum, whole kidney, pancreas, spleen, liver, heart, incisors (pulpless crowns), mandibular condyle, mandibular bone (ramus and part of corpus), tibia epiphysis and tibia diaphysis (bone marrow removed) were taken using polyethylene or polyethylene-covered instruments, placed in weighed Pyrex tubes and weighed immediately. Thereafter, the samples were dried in an oven for 4 to 6 hours at 100°C and then overnight at 150°C. They were transferred to a muffle oven and the temperature was slowly raised and maintained at 550°C for a second night. When they had cooled to room temperature, the ash weights were recorded and the ash dissolved in 0.5 ml HCl 10.5 % overnight. All samples were then diluted with 2.0 ml 3x-distilled water (BERGMAN et coll. 1974).

As 49 % of the disintegrations of ^{65}Zn result in the emission of 1.116 MeV γ-rays and 100 % of the disintegrations of ^{85}Sr in the emission of 0.514 MeV γ-rays (LEDERER et coll. 1968), a 7.6 cm $\times$ 7.6 cm NaI(Tl) well scintillation detector with a two channel analyzer (Picker Dual Channel Analyzer), one channel calibrated to count at each of these two energy levels, was used to measure the ^{65}Zn and ^{85}Sr activities in the samples. Known amounts of ^{65}Zn, ^{85}Sr and the injection solution in separate tubes were diluted to the same volume, 2.5 ml, to assure comparable counting geometry and were measured as references on each occasion. The background counting rate was also measured on each occasion and subtracted from the counting rate for each sample and reference. As the annihilation γ-rays (0.511 MeV) and some of the Compton-scattered 1.116 MeV γ-rays from ^{65}Zn are also counted in the 0.5 MeV channel, the counting rate in this channel due to the ^{85}Sr γ-rays must be separated from the ^{65}Zn counting rate. The counting rate (cpm) in the 0.5 MeV channel due

to the ^{85}Sr in a tissue ash or injection solution sample was calculated using the ratio (R) of the cpm in the 0.5 MeV channel to the cpm in the 1.1 MeV channel for the ^{65}Zn reference sample:

$$\text{sample } ^{85}\text{Sr cpm at 0.5 MeV} =$$
$$(\text{sample total cpm at 0.5 MeV}) - R \cdot (\text{sample total cpm at 1.1 MeV}).$$

The ^{85}Sr in the samples was not sufficient to cause a significant counting rate above background in the 1.1 MeV channel.

After the ^{65}Zn and ^{85}Sr activities had been measured, the samples were further diluted with HCl 2.2 % as needed and the zinc concentrations determined by atomic absorption spectrophotometry using a Unicam SP 90 spectrophotometer at 213.9 nm with a zinc lamp. For each of the mineralized tissue samples a further absorption measurement was made at 210 nm using the same lamp and subtracted from that at 213.9 nm in order to correct for the absorption due to the high calcium concentration (BERGMAN et coll. 1974).

The relative activities (RA) of ^{65}Zn and ^{85}Sr in the samples were defined and calculated as follows:

$$\text{RA} = \frac{\text{cpm/g fresh weight of the sample}}{\text{cpm injected/g body weight}}$$

In the presentation of the data, these values are multiplied by 100 to obtain the RA in per cent. The ^{65}Zn specific activity (SA) in each sample was obtained by dividing the ^{65}Zn RA by the zinc concentration (mg Zn/mg fresh weight of the sample). The ^{85}Sr SA in serum, viscera and heart were obtained by dividing the ^{85}Sr RA by the respective average calcium concentrations (g Ca/g fresh weight of the sample) obtained in a separate investigation of four female rats of the same age using the same sample preparation as above and operating the SP 90 at 422.7 nm with a calcium lamp. The ^{85}Sr SA in the mineralized tissue samples were calculated by dividing the ^{85}Sr RA in each sample by its ash weight/fresh weight ratio and by the average ash weight calcium concentrations (g Ca/g ash) obtained from these same four rats.

The calculations of the RA and SA and the means and standard deviations for all data as well as the variance and least squares analyses were performed on an electronic computer (Control Data 3200, Umeå Datacentral) or a programmable calculator (Canon Canola 167P) using standard programs. In the presentation of the results of statistical tests, the following levels of significance are used:

$0.05 < p$	not significant	
$0.01 < p \leqslant 0.05$	nearly significant	*
$0.05 < p \leqslant 0.01$	significant	**
$p \leqslant 0.001$	highly significant	***

where p is the probability of incorrectly rejecting the null hypothesis.

Results

The means and standard deviations of the fresh weight zinc concentrations in the eleven samples from all 48 rats are presented in Table 1. For the concentrations in serum, viscera and heart, the coefficients of variation ($100 \cdot SD/\bar{x}$) within the individual groups ($n=4$) averaged 9 per cent and were largest for pancreas and liver and least for spleen. These concentrations were quite stable between 6 and 9.5 weeks of age and the analysis of variance on the mean concentrations in these samples from the twelve groups was significant only for those in the liver, the mean concentration in which was relatively low at 6 days following injection. The coefficients of variation for the concentrations in the mineralized tissues also averaged 9 per cent and were highest for mandibular condyle. The zinc concentrations in mandibular condyle and mandibular bone increased slightly and that in tibia epiphysis moderately with increasing age. The analyses of variance were significant or highly significant for the concentrations in these three samples but not for those in the incisors or tibia diaphysis.

The means and standard deviations of the ash weight/fresh weight ratios and the ash weight zinc concentrations in the mineralized tissues are also presented in Table 1. The coefficients of variation for the ash weight/fresh weight ratios averaged 6 per cent and were greatest for mandibular condyle. In the bone samples but not the incisors, the ratios increased with increasing age and the analyses of variance were significant or highly significant. The coefficients of variation for the ash weight zinc concentrations averaged 8 per cent and were largest for mandibular condyle and incisors. These concentrations were stable throughout the experimental period and the analyses of variance were nearly significant only for those in tibia epiphysis.

The body weights of these rats increased with increasing age during the experimental period. They averaged 130 g ($SD=11$ g, $n=48$) at the time of injection (6 weeks old) and the last group killed 24 days later weighed 194 g ($SD=12$ g, $n=4$). The analysis of variance on the mean body weights of the individual groups ($n=4$) was highly significant ($F=13.06^{***}$).

The mean ^{85}Sr relative activities (RA) in the serum, viscera, heart and mineralized tissue samples at twelve different times following injection are presented in Fig. 1. The groups at 0.25, 1, 6, 12 and 16 days following injection are composed of only three rats because both the ^{85}Sr and ^{65}Zn activities in all eleven samples from the rat which was eliminated were very low compared to those in the remaining rats in the group, indicating that an error had been made in the injection. The coefficients of variation for the ^{85}Sr RA in the serum averaged 40 % (range 10 to 133 %) and were greatest for the data from later post-injection times. The coefficients of variation for the ^{85}Sr RA in viscera and heart were very large, due to the low amount of ^{85}Sr activity in these tissues relative to the ^{65}Zn activity, and largest for the RA from later times post-injection. The activities in these tissues beyond 3 days following injection are not presented as the coefficients of variation were greater than 100 per cent and a

Table 1

The fresh weight zinc concentrations in ppm (μg/g) in the eleven samples from 6- to 9½-week old females rats killed in groups of four at twelve different times after injection with ^{65}Zn at 6 weeks of age. The ash weight/fresh weight ratios and the ash weight zinc concentrations in the mineralized tissue samples are also given. The means are those for all 48 rats and the standard deviations are those for the individual groups (within) and for all 48 rats (total) obtained in the analysis of variance on the twelve sacrifice groups, the results of which are presented under F with the levels of significance. To the right are given the average calcium concentrations obtained in a separate experiment and used in the calculation of the ^{85}Sr specific activities (SA)

	$\bar{x}$	S.D. within	S.D. total	F	$\bar{x}$
	Zn ppm fresh weight				$10^3 \cdot$g Ca/g fresh weight
Serum	1.34	0.14	0.15	1.61	0.1035
Kidney	23.0	1.3	1.3	0.97	0.0692
Pancreas	22.4	3.5	3.4	0.75	0.1131
Spleen	22.2	0.6	0.6	1.42	0.0451
Liver	28.0	3.0	3.9	3.68**	0.0389
Heart	17.6	1.4	1.4	0.59	0.0404
Incisors	89.8	7.3	7.7	1.47	
Mand. condyle	117.2	13.8	16.6	2.92**	
Mand. bone	135.2	10.4	12.7	3.12**	
Tib. epiphysis	100.1	7.5	18.5	22.50***	
Tib. diaphysis	161.9	14.0	15.2	1.76	
	Ash weight/fresh weight				
Incisors	0.679	0.031	0.030	0.71	
Mand. condyle	0.289	0.027	0.035	3.91***	
Mand. bone	0.439	0.020	0.033	8.83***	
Tib. epiphysis	0.218	0.016	0.034	15.43***	
Tib. diaphysis	0.524	0.028	0.035	3.39**	
	Zn ppm ash weight				g Ca/g ash weight
Incisors	132.3	11.6	11.2	0.71	0.345
Mand. condyle	406	40	41	1.16	0.369
Mand. bone	308	20	21	1.64	0.369
Tib. epiphysis	459	23	27	2.49*	0.366
Tib. diaphysis	309	24	23	0.91	0.376

large number of the calculated activities were negative, which is possible with the method used for separating the ^{85}Sr from the ^{65}Zn activity. The coefficients of variation averaged 17 per cent for the RA in the incisor crowns (range 5 to 51 %) and 11 per cent for those in the bone samples (range 3 to 36 %). The bar in Fig. 1 and subsequent figures represents a coefficient of variation of 10 per cent.

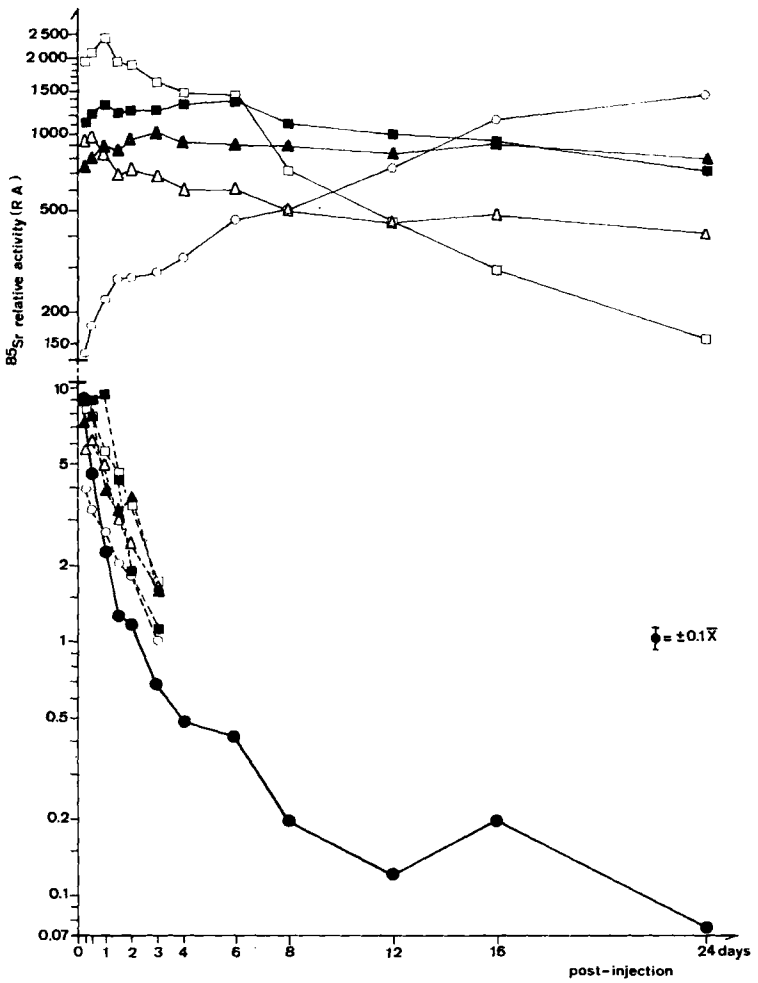

Fig. 1. The mean ^{85}Sr relative activities (n = 4; for 0.25, 1, 6, 12 and 16 days, n = 3) for the 11 samples from 6-week old rats. The bar represents a coefficient of variation (100·SD/$\bar{x}$) of 10 per cent.
●—● serum, □---□ kidney, ■---■ pancreas, △---△ spleen, ▲---▲ liver, ○---○ heart, □—□ mandibular condyle, ■—■ mandibular bone, △—△ tibia epiphysis, ▲—▲ tibia diaphysis, ○—○ incisors.

The means of the estimated ^{85}Sr specific activities in these samples are presented in Fig. 3. The coefficients of variation for the SA in serum, viscera and heart were necessarily the same as those for the RA in the respective samples. The coefficients for the SA in incisor crowns averaged 19 per cent and those for the bones averaged 12 per cent, thus slightly higher than the coefficients of variation for the corresponding RA.

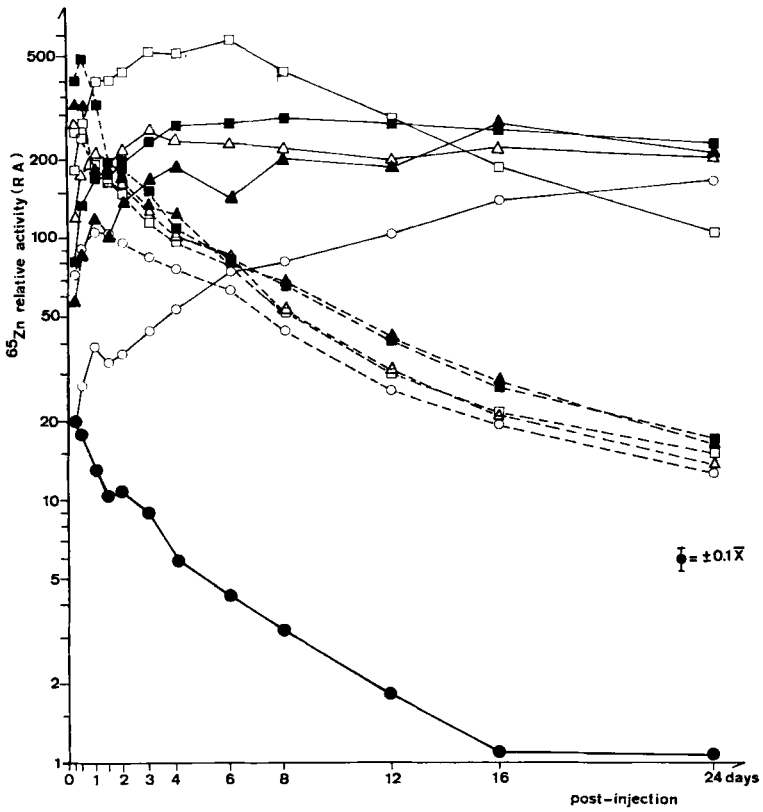

Fig. 2. The mean ^{65}Zn relative activities (n = 4; for 0.25, 1, 6, 12 and 16 days, n = 3) for the 11 samples from 6-week old rats. The bar represents a coefficient of variation (100·SD/$\bar{x}$) of 10 per cent. (Symbols as in Fig. 1.)

The ^{85}Sr RA in serum was relatively low and the SA high at 0.25 day. The activity decreased extremely rapidly during the first 1.5 days following injection and much more slowly thereafter. The course of the serum SA during the short period from 0.25 through 1.5 days (Fig. 3) may be approximated by the straight line $SA_t = 1089 \cdot e^{-1.542 \cdot t}$. Plotting the serum activity on log − log scales (Fig. 5) reveals a straight line course from 0.25 through 4 days, $SA_t = 205.4 \cdot t^{-1.051}$. The line best fitting the data from 0.25 through 24 days has a slope (exponent) of −0.991. These and subsequent lines were obtained by the least squares method on the logarithms of the data from the time period against the time post-injection or the logarithm of the time.

The ^{85}Sr RA in the viscera were less than that in serum at 0.25 day following injection and the SA, except for that in pancreas, were greater than the SA in serum. The activities in these organs decreased more slowly than that in serum during the next few days. The SA in pancreas was nearly equal to that in serum at 0.25 day and,

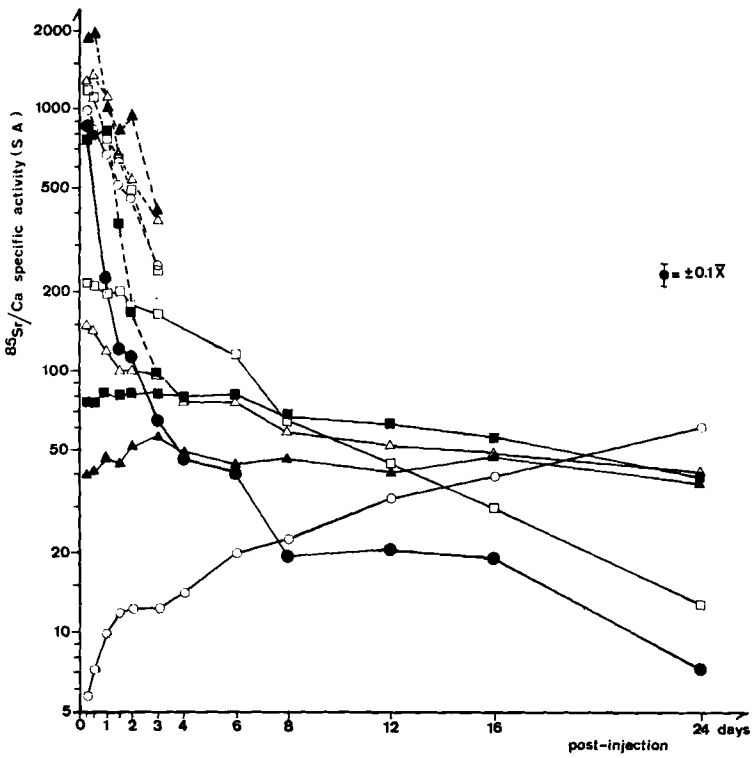

Fig. 3. The mean ^{85}Sr specific activities (n = 4; for 0.25, 1, 6, 12 and 16 days, n = 3) for the 11 samples from 6-week old rats. For serum, viscera and heart, the SA were obtained by dividing the RA (Fig. 1) by the mean fresh weight calcium concentrations in Table 1. For the mineralized tissue samples, the RA were divided by ash weight/fresh weight ratio in the sample and the appropriate mean ash weight calcium concentration given in Table 1. The bar represents a coefficient of variation ($100 \cdot SD/\bar{x}$) of 10 per cent. (Symbols as in Fig. 1.)

beginning at 1 day, decreased as rapidly as the activity in serum decreased shortly earlier.

For each of the bone samples, the ^{85}Sr RA and SA curves differ somewhat at the earlier times following injection. The initial rises in the RA were not present in the SA curves except that for tibia diaphysis. The mandibular condyle SA decreased exponentially from 0.25 through 6 days with a slope (exponent) of -0.113. Between 6 and 8 days the rate of loss of activity was accelerated and then returned to approximately the original rate (-0.102). After an initial period of rapid loss, the RA and SA in tibia epiphysis decreased more slowly through 8 days post-injection and even more slowly through the remainder of the experimental period. The later rate of decrease was greater for the SA than for the RA in tibia epiphysis. After initial

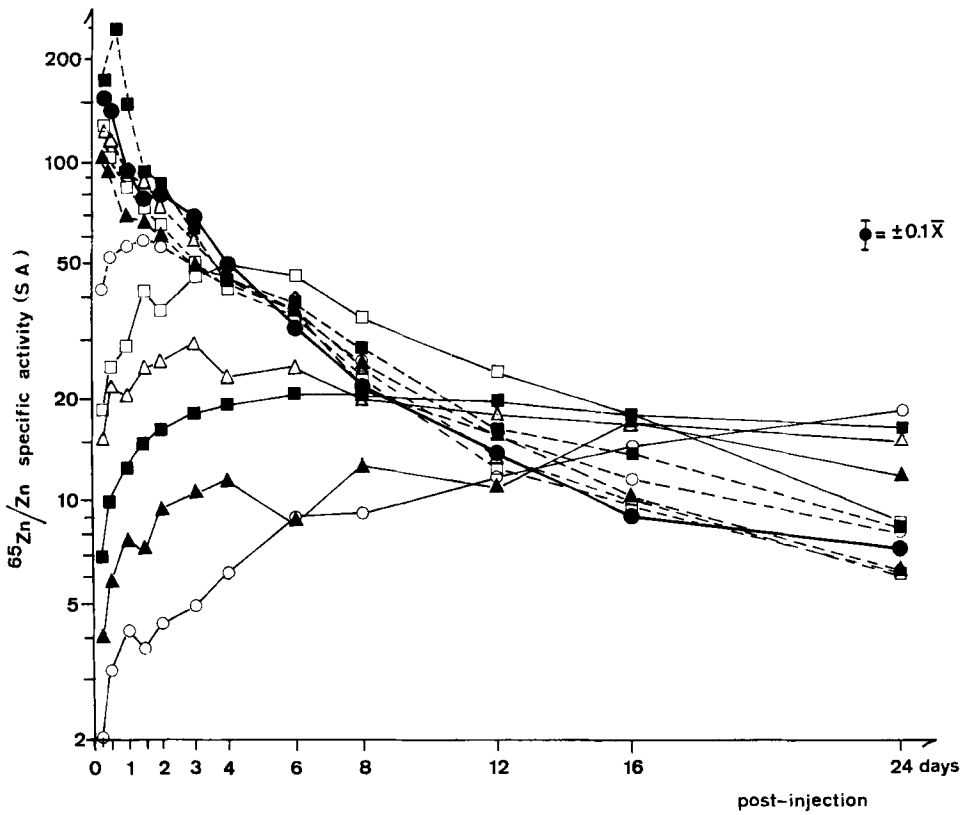

Fig. 4. The mean ^{65}Zn specific activities (n = 4; for 0.25, 1, 6, 12 and 16 days, n = 3) for the 11 samples from 6-week old rats. The SA in each sample was obtained by dividing the RA by the zinc concentration. The means of the zinc concentrations used to calculate these values are presented in Table 1. The bar represents a coefficient of variation (100·SD/$\bar{x}$) of 10 per cent. (Symbols as in Fig. 1.)

periods of smaller increases, the RA and SA in mandibular bone and tibia diaphysis decreased very slowly. The activity in the incisor crowns was the lowest of those in the mineralized tissues at 0.25 day and increased exponentially from the third day onward.

The means of the ^{65}Zn RA in these same serum, viscera, heart and mineralized tissue samples are presented in Fig. 2. The coefficients of variation averaged 11 per cent for all samples and killing times (range 1 to 37 %). The individual sample zinc concentrations were used to calculate the SA, the means of which are presented in Fig. 4. The coefficients of variation for the SA averaged 11 per cent for all samples and killing times or the same as the average coefficient of variation for the RA.

At 0.25 day, the serum ^{65}Zn RA was lower than all but that in incisors and the serum SA was higher than all but that in pancreas. The serum activity decreased at a moderately rapid rate through 1.5 days, increased slightly at 2 days and then

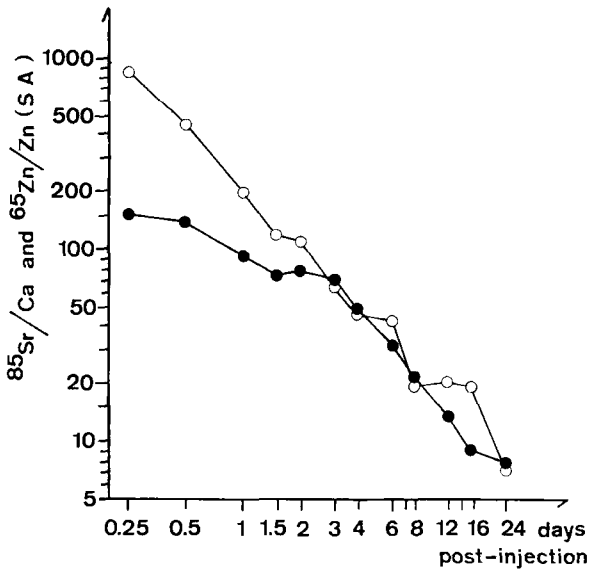

Fig. 5. The mean ^{85}Sr ○ and ^{65}Zn ● specific activities in the serum. Both the SA and the time axes are logarithmic. The data are the same as those in Figs 3 and 4 respectively.

decreased at a rate slower than the initial rate. The coefficients of variation at 2, 3 and 4 days were relatively large. The course of the serum SA during the periods from 0.25 through 1.5 and from 2 through 8 days (Fig. 4) may be approximated by two straight lines $SA_t = 183.7 \cdot e^{-0.613 \cdot t}$ and $SA_t = 125.7 \cdot e^{-0.223 \cdot t}$, respectively. Plotting the serum SA on log − log scales (Fig. 5) reveals a straight line course between 3 and 24 days, $SA_t = 228.5 \cdot t^{-1.118}$ or a slightly faster rate of loss than for ^{85}Sr.

The RA in the viscera were on the average 15 times that in serum at 0.25 day. The SA in pancreas was slightly greater than that in serum and, after increasing to an even higher level at 0.5 day, decreased approximately parallel to the serum SA from 1.5 day. The SA in kidney, spleen and liver were slightly below the serum SA at 0.25 day and, decreasing, slowly approached the serum SA and were equal to it at 5 days after injection. The RA in heart is one half and the SA one fourth of the RA and SA, respectively, in serum at 0.25 day and increasing. The SA in heart reached a peak at 1.5 days and, decreasing, slowly approached the serum SA. After 4 days, the SA in the heart behaved much the same as those in the viscera, following the serum SA in its downward course.

For each of the bone samples, the ^{65}Zn RA and SA differ only in that the RA rose slightly more rapidly or decreased more slowly than the SA. For the mandibular condyle this difference was very small while the difference in the rate of decrease of the RA and SA in tibia epiphysis at later times was most marked. The SA in mandibular condyle was highest of those in the mineralized samples at 0.25 day and increased until it crossed the serum SA curve at 4 days. After a short period with a high rate of decrease between 6 and 8 days, the SA decreased exponentially

($e^{-0.0855 \cdot t}$) at a rate less than that for serum. The SA in tibia epiphysis reached a peak by the third day and, while decreasing at a rate slower than that for the serum SA, crossed this curve at 9 days and decreased at a very slow rate thereafter. The mandibular bone SA reached its peak between 8 and 12 days, at which time it crossed the serum SA curve, and then decreased exponentially at a very slow rate. The SA in tibia diaphysis had an irregular course at times. The SA appeared to increase through 6 to 8 days and then stabilize at the 8 day level. The SA in incisors was initially the lowest and increased exponentially from the sixth day onward.

At later times the courses of the ^{65}Zn RA in the bone samples approached those of the ^{85}Sr RA. In incisors, the courses of the ^{85}Sr and ^{65}Zn RA were very much alike, the major difference occurring at early times post-injection when the ^{65}Zn RA increased more rapidly than the ^{85}Sr RA.

Estimates of the exchangeable pool sizes E and the accretion rates A for both ^{85}Sr and ^{65}Zn in the bone samples have been made using the equation of BAUER et coll. (1955 a)

$$R_t = E \cdot S_t + A \cdot \int_0^t S_t \cdot dt,$$

where R_t is the retention of activity (RA) in the sample and S_t is the specific activity (SA) in serum at time t. The serum activities are assumed to decrease exponentially through 1.5 days, $S_t = S_0 \cdot e^{-K_S \cdot t}$, thus

$$R_t = S_0 \cdot \left[E \cdot e^{-K_S \cdot t} + \frac{A}{K_s} \cdot (1 - e^{-K_S \cdot t}) \right].$$

The E- and A-values were calculated using each of the six possible pairs of data from 0.25, 0.5, 1.0 and 1.5 days and solving two simultaneous equations. The calculated value for E increases and that for A decreases as much as two- or three-fold as data from the later of these times are used in the equation. In Table 2 are presented the calculated values for the data from 1.0 and 1.5 days as they give the highest value for E and the lowest for A. The values are based on 1 gram fresh weight of bone. The data point at 1 day for ^{65}Zn in mandibular condyle and at 1.5 day for ^{65}Zn in tibia diaphysis were not used as they give values which are very much different from the remaining points and they appear to be extreme points compared to the general trend in these samples.

As it is believed that the retention in the bone samples by 6 days reflects almost exclusively the accretion phase as it is estimated by the equation of BAUER et coll. and that little resorption should have occurred by this time, the equation may be simplified to

$$R_t \simeq A \cdot \int_0^t S_t \cdot dt.$$

A further estimate of the value for A may be made by dividing the retention at 6 days by an estimate of the integral of the serum curve from 0 to 6 days obtained

Table 2

The values for the exchangeable pool size E and the accretion rate A for calcium (^{85}Sr) and zinc (^{65}Zn) in the bone samples calculated using the equation of BAUER *et coll. (1955a) and the data from 1 and 1.5 days. The retention R_6 in the sample at 6 days has been divided by the graphically estimated time integral of the serum specific activity S_t as a further estimate of A. The values in per cent are obtained by dividing by the appropriate calcium or zinc concentrations. All values are based on 1 gram fresh weight of bone sample*

	BAUER et coll.				$R_6/\int_0^6 S_t \cdot dt$	
	E	*A*	*E*	*A*	*A*	*A*
^{85}Sr	g Ca	g Ca·day^{-1}	% Ca	% Ca·day^{-1}	g Ca·day^{-1}	% Ca·day^{-1}
Mand. condyle	0.0533	0.0205	54.6	16.0	0.0145	13.9
Mand. bone	0.0191	0.0156	12.4	10.1	0.0141	9.0
Tib. epiphysis	0.0162	0.0079	23.1	11.3	0.0064	8.8
Tib. diaphysis	0.0083	0.0121	1.2	6.3	0.0093	4.8
^{65}Zn	mg Zn	mg Zn·day^{-1}	% Zn	% Zn·day^{-1}	mg Zn·day^{-1}	% Zn·day^{-1}
Mand. condyle[a]	0.0089	0.0191	7.4	15.9	0.0135	11.7
Mand. bone	0.0076	0.0071	5.8	5.4	0.0065	5.0
Tib. epiphysis	0.0087	0.0075	10.1	8.7	0.0054	6.0
Tib. diaphysis[b]	0.0023	0.0072	1.4	4.5	0.0043[c]	2.7

[a] data from 0.5 day replaces 1 day
[b] data from 0.5 day replaces 1.5 days
[c] data from 8 and 0–8 days respectively

graphically. These values are also presented in Table 2. Each of the values for *E* and *A* in Table 2 has been divided by the appropriate average calcium or zinc concentration to obtain the value in per cent.

The *A*-values for both ^{85}Sr and ^{65}Zn as calculated by the equation of BAUER et coll. were between 10 and 60 per cent higher than the corresponding *A*-values estimated graphically. The *E*- and *A*-values in per cent for both isotopes in the cortical bone of the tibia diaphysis and the predominantly cortical bone of the mandible were less than the corresponding values for the endochondral bone of the mandibular condyle or the calcifying cartilage of the tibia epiphysis. The fraction of the zinc which was rapidly exchangeable (*E*%) was in each case less than the fraction of calcium, the difference between the two in mandibular condyle being the greatest. The relative rates of 'accretion' (*A*%) of zinc in the bone samples were in each case less than the respective relative rates of accretion of calcium, and in the cortical bone samples they were much less.

Due to the effects of eruption and attrition on the data from the incisors, the *E*- and *A*-values cannot be calculated using the equation of BAUER et coll. The reten-

tion at 6 days divided by the time integral of the serum SA from 0 to 6 days was 0.0048 for ^{85}Sr and 0.0018 for ^{65}Zn. Dividing these values by the calcium and zinc concentrations, respectively, in incisors gave values of 2.05 ($\%\cdot day^{-1}$) for calcium and 2.00 for zinc.

Discussion

The methods and method errors have been discussed at length previously (BERGMAN et coll. 1974, BERGMAN 1970). The coefficients of variation ($100\cdot SD/\bar{x}$) for the wet weight and ash weight zinc concentrations and ash weight/fresh weight ratios in most samples are less than 10 per cent (Table 1) as they were in the report of the zinc concentrations during growth (BERGMAN et coll. 1974). The coefficients of variation for the ^{85}Sr activities in serum and bones average 40 and 13 per cent, respectively. The coefficients of variation for the ^{65}Zn relative and specific activities (RA, SA) average 11 per cent.

While the major source of random error may be the biologic variation, other sources are also important. The size of the sample taken can affect its relative morphology, due to the heterogenicity of several of these tissues, and may affect the zinc, ash (calcium) and nuclide concentrations. The relative success of the injection will dramatically effect the RA and SA in all samples. This is apparent in some rats in which both the ^{85}Sr and ^{65}Zn activities in all eleven samples were much lower than in those from the remaining rats in the same sacrifice group. These data were eliminated. Smaller errors in the injections may go undetected and thus increase the total random error. The large errors in the ^{85}Sr activities in the viscera were anticipated from the method used for separating the ^{85}Sr from the total activity in the 0.5 MeV channel. The relative amounts of the nuclides injected were chosen in order to make the relative concentrations in the serum and mineralized tissues as favorable as possible at the expense of those in the viscera. The ^{85}Sr RA and SA in the viscera are sufficiently reproducible at early times following injection such that a pattern can be discerned, and this is sufficient to the present purposes.

The fresh weight and ash weight zinc concentrations and the ash weight/fresh weight ratios in these 6 to 9.5 weeks old rats (Table 1) compare well with those for 6 and 8 weeks old rats in the previous report on the variation of the zinc concentrations with age (BERGMAN et coll. 1974). The relationship of the zinc concentration to the mineral concentration in the tibia epiphysis during active mineralization found was confirmed by the present data.

That these animals were in an active growth period is indicated by the increase with increasing age in both the body weights and the ash weight/fresh weight ratios in the mineralized tissue samples. While the fresh weight zinc concentrations in the serum, heart and the viscera, with the exception of the liver, and the ash weight zinc concentrations in the mineralized tissues do not vary significantly during the experimental period, the ash weight/fresh weight ratios (fresh weight calcium concentrations) and fresh weight zinc concentrations do increase in most of the mineralized

samples causing the concentrations of calcium and zinc in the animals as a whole to increase. These increases are the cause of the divergence of the RA and SA curves for each mineralized tissues at later post-injection times.

In order to test the validity of the nuclide retention data and to be able to interpret them, a separate experiment was made on a 7-week old female rat. The fresh weight and ash weight concentration of calcium and zinc as well as the ash weight/fresh weight ratio in the animal as a whole were determined (10.4 mg Ca, 29.6 μg Zn and 37.2 mg ash per g body weight, and 280 mg Ca and 795 μg Zn per g ash). As well over 99 per cent of the calcium in the body is located in the bones and teeth and the ash weight calcium concentrations in these tissues appear to be relatively stable (a value of 0.37 g Ca/g ash was used which is representative for the values in cortical bone given in Table 1 obtained by the same method as was used in this experiment), the calculated part of the total body ash representing bones and teeth is 75 per cent. If the ash weight/fresh weight ratios in mineralized tissues of rats of this age average 0.40, approximately 7 per cent of the rat's body weight is mineralized tissue. Thus, if losses to excretion were negligible and all the ^{85}Sr was accumulated in the mineralized tissues, the maximum average expected ^{85}Sr RA in per cent would be 1 400, or fourteen times that injected per gram body weight. The observed RA in the bone samples at 0.25 day range from 773 to 1 967 per cent. If, instead, the bones and teeth of rats of this age were to comprise 10 per cent of the body weight, a generally accepted value, the observed ^{85}Sr RA would be too high as they should not, with very few exceptions, exceed 1 000 per cent.

The expected ^{65}Zn RA were much lower due to the more even distribution of zinc in the body. If the 7 per cent of the body which is mineralized tissue contains an average of 135 ppm (μg/g) zinc, then approximately one third of the zinc in the rat is located in the bones and teeth, and the remaining tissues of the body should have an average fresh weight zinc concentration of 22 ppm. If all zinc were equilibratable and excretion negligible, the average expected RA in per cent in the mineralized tissues would be 470 per cent and the average in the remaining tissues would be 70 per cent. The RA in the bones at 0.25 day are between 50 and 200 per cent and only the RA in mandibular condyle reaches as high as 470 per cent. If the zinc in bones and teeth were unavailable for exchange, the average expected RA in the samples other than bone should not exceed 150 per cent or 1.5 times that injected per gram body weight. The average RA in the viscera is twice this value at 0.25 day despite the fact that the bones had accumulated significant activity by this time. Thus a sizable fraction of the zinc in some of the tissues of the body in addition to that in bone must exchange slowly with the ^{65}Zn in serum if the RA in viscera are to be as large as 300 per cent. If the heart can be used as approximately representative for skeletal muscle as well in this respect (BERGMAN & SÖREMARK 1968), then the approximately one third of the total body zinc in the skeletal muscle may be a large part of this slowly exchanging fraction. The relatively large fraction of the body's zinc located in the hair also accumulates ^{65}Zn at a very slow rate (BERGMAN & SÖREMARK 1968).

The accuracy of the ^{85}Sr and ^{65}Zn SA in serum are very important to the accuracy of the calculated *E*- and *A*-values as both values vary inversely proportionally to the respective serum SA. The average serum calcium concentration used in calculating the ^{85}Sr SA is 0.000104 g Ca/g serum which is approximately equivalent to 10 mg % and close to the generally accepted value. This concentration is expected to be very stable. The zinc concentrations in serum may be 15 to 20 per cent too low due to losses of zinc to the glass tubes during ashing (BERGMAN et coll. 1974). This systematic error would cause the serum ^{65}Zn SA to be approximately 20 per cent too high and the *E*- and *A*-values to be 15 to 20 per cent too low. Thus the *E*- and *A*-values should be multiplied by a factor of 1.2 to obtain more accurate values.

The *A*-values for mandibular condyle are 16 per cent for both calcium (^{85}Sr) and zinc (adjusted value for zinc 19 per cent) which indicate that the isotope fronts should reach the resorption zone in 5 to 7 days. The marked decreases in the activities of both isotopes between 6 and 8 days indicate that the activity fronts have reached the resorption zone by this time. The ^{85}Sr RA decreases relatively more than the ^{65}Zn RA at this time. As the specific activity of the ^{85}Sr in the bone which is being accreted at the time of the injection should be higher relative to the remainder of the retention than the specific activity of the ^{65}Zn front is in relation to the remainder of the ^{65}Zn in the condyle, this may be the cause of the difference in the relative loss of the two isotopes between 6 and 8 days. The agreement of the RA in the incisors divided by the time integral of the serum SA and the concentration of the element also indicates that zinc (^{65}Zn) is being accumulated at the same relative rate as calcium (^{85}Sr). These values cannot be used as accretion rates as the eruption rate dominates the shape of the retention curves. Both these observations appear to support the hypothesis that zinc is being 'accreted' in bones and teeth.

The similarities in the courses of the ^{85}Sr and ^{65}Zn RA in the bone samples at later times post-injection may indicate that similar processes dominate the loss of both isotopes. Resorption is expected to play a dominant role at these times. This might be interpreted as further indicating that zinc, like calcium and strontium, is being 'accreted' or moved from an exchangeable pool to one which is relatively unavailable for exchange. It is as yet unknown whether or not zinc replaces calcium in the lattice of the apatite crystals in bones and teeth, but zinc may be 'buried' in newly accreted bone and tooth mineral even if it is located in the organic matrix or the hydration layer of the crystals (BRUDEVOLD et coll. 1963, HAUMONT & MCLEAN 1966, JOWSEY & ORVIS 1967). It was on this assumption that the equation of BAUER et coll. was applied to the ^{65}Zn as well as the ^{85}Sr data.

The increasing *E*-values and decreasing *A*-values as data from later times post-injection are used in the calculations have been observed previously by many authors (MARSHALL 1969). Comparing the lowest *A*-values calculated using the equation of BAUER et coll. with those obtained by dividing the retention at 6 days by the integral of the serum SA to 6 days, the *A*-values are expected to decrease even more if data from times later than 1.5 days are used in the equation. In an effort to quantitatively

describe the behaviour of ^{65}Zn in heart muscle and to calculate E- and A-values which obtain at all times post-injection, an equation was derived to describe the retention with time in a slowly exchanging compartment (see Appendix), incorporated in the equation of BAUER et coll. and used with this data. This 3-compartment equation has its parallel in the analogue technique used by WENDEBERG (1965) and others for ^{47}Ca and ^{85}Sr and the digital equivalent used by MCINTOSH & LUTWAK-MANN (1972) for ^{65}Zn.

The equation for the retention R_{L_t} in a slowly exchanging compartment, assuming that the course of the serum SA is initially exponential, $S_t = S_0 \cdot e^{-K_s \cdot t}$, is

$$R_{L_t} = S_0 \cdot L \cdot \frac{K_L}{K_L - K_s} \cdot (e^{-K_s \cdot t} - e^{-K_L \cdot t})$$

where S_0 is the initial serum SA (extrapolated), L is the size of the slowly exchanging compartment, and K_s and K_L are the rate constants of the serum and compartment respectively. As the earlier courses of the serum SA for both ^{85}Sr and ^{65}Zn in the data presented here can be approximated by single exponentials through 1.5 days post-injection, the full equation for the retention R_t in a sample containing rapidly exchanging (E), more slowly exchanging (L) and accreting (A) fractions becomes

$$R_t = S_0 \cdot \left[E \cdot e^{-K_s \cdot t} + L \cdot \frac{K_L}{K_L - K_s} \cdot (e^{-K_s \cdot t} - e^{-K_L \cdot t}) + \frac{A}{K_s} \cdot (1 - e^{-K_s \cdot t}) \right]$$

Unfortunately this equation cannot be solved directly for E, L, K_L and A using simultaneous equations. Under certain circumstances, as for example when the accreted fraction of the activity in a sample can be assumed to be very small relative to the activity in the exchangeable pools E and L, K_L can be solved directly using the ratios of the sample and serum SA from the times when the sample activity is at its peak and after it has assumed a constant ratio to the serum SA (see Appendix). If accretion is likely to be playing a dominant role in the retention of activity, as it does for ^{85}Sr and ^{65}Zn in the bone samples, it is still possible to solve the full equation by making successive approximations to K_L and then solving three simultaneous equations for E, L and A. This method was applied to the same data as were used in the equation of BAUER et coll. A programmable calculator (Canon Canola 167P) was used to solve the equations.

The results of the method of successive approximations on the data from the bone samples are presented in Table 3. When K_L is chosen such that the E component is insignificantly small, that is, only the more slowly exchanging compartment L and accretion A are operative, the accretion rates calculated using the data from three of the four killing times between 0.25 and 1.5 days are with few exceptions at least as small as the smallest calculated with the equation of BAUER et coll., and thus closer to the values obtained graphically at 6 days, and the predicted retention at the fourth point falls within the range $\bar{x} \pm 2 \cdot SE_{\bar{x}}$ of the observed RA for both nuclides in each of the bone samples.

Table 3

The values for the size of the slowly exchanging compartment L with an exchange rate K_L and either the accretion rate A or the size of the rapidly exchangeable pool E for calcium (^{85}Sr) and zinc (^{65}Zn) in the bone samples calculated using the modified equation for retention and the data from 0.25, 1.0 and 1.5 days. The values to the left were obtained when the rapidly exchangeable pool E was of insignificantly small size and those to the right when the accretion rate A was at or near 0. All values are based on 1 gram fresh weight of bone sample

	E inoperative					*A* inoperative				
	K_L	L	A	L	A	E	K_L	L	E	L
^{85}Sr	day^{-1}	g Ca	g Ca·day^{-1}	% Ca	% Ca·day^{-1}	g Ca	day^{-1}	g Ca	% Ca	% Ca
Mand. condyle	2.3	0.0408	0.0154	40.3	15.2	0.0073	0.96	0.0740	7.2	73
Mand. bone	4.1	0.0136	0.0153	8.8	9.9	0.0071	0.44	0.0591	4.6	38
Tib. epiphysis	5.3	0.0120	0.0078	17.3	11.3	0.0082	0.52	0.0302	11.9	44
Tib. diaphysis	7.9	0.0067	0.0120	3.5	6.2	0.0061	0.11	0.1304	3.2	68
^{65}Zn	day^{-1}	mg Zn	mg Zn·day^{-1}	% Zn	% Zn·day^{-1}	mg Zn	day^{-1}	mg Zn	% Zn	% Zn
Mand. condyle[a]	4.0	0.0099	0.0180	8.8	16.1	0.0032	0.52	0.0648	2.9	58
Mand. bone	2.0	0.0089	0.0056	6.8	4.3	0.0011	0.64	0.0275	0.8	21
Tib. epiphysis	4.6	0.0079	0.0074	9.4	8.8	0.0041	0.52	0.0267	4.9	32
Tib. diaphysis[b]	5.6	0.0023	0.0070	1.5	4.5	0.0011	0.48	0.0212	7.1	14

[a] data from 0.5 day replaces 1.0 day
[b] data from 0.5 day replaces 1.5 day

In effect what is done by allowing the E-value to approach 0 is to replace the rapidly exchanging compartment E with the less rapidly exchanging compartment L. The equation used for the retention is then

$$R_t = S_0 \cdot \left[L \cdot \frac{K_L}{K_L - K_s} \cdot (e^{-K_s \cdot t} - e^{-K_L \cdot t}) + \frac{A}{K_s} \cdot (1 - e^{-K_s \cdot t}) \right]$$

The equation of BAUER et coll. is a simplification of the above equation, the assumption having been made that K_L is so much larger than K_s that

$$L \cdot \frac{K_L}{K_L - K_s} \cdot (e^{-K_s \cdot t} - e^{-K_L \cdot t}) \approx L \cdot e^{-K_s \cdot t}$$

The results indicate that this assumption may not be valid. Assuming that the retention equation above is a more accurate description of retention than the equation of

BAUER et coll., the A-value calculated by solving this equation for two unknowns ($E = L \cdot K_L/(K_L - K_s)$ and A) will decrease and become more accurate at later times, while the E-value will increase and will eventually be over-estimated by a factor of $K_L/(K_L - K_s)$.

MARSHALL (1969) uses the decreasing A-values as evidence for the existence of very slowly exchanging or expanding compartments and argues that the straight line course of the serum activities on log − log scales with a slope near −1 (Fig. 5) is further evidence of the existence of such compartments. In an attempt to detect whether or not the existence of very slowly exchanging compartments was consistent with the present data, further approximations to K_L were made for each of the bone samples and isotopes. Using the same data, the K_L-values were decreased until the accreted fraction was no longer operative (Table 3). The predicted value for the data point not used in the calculation in each case falls within the range $\bar{x} \pm 2 \cdot SE_{\bar{x}}$ of the measured RA. For ^{85}Sr, the fit to the data is improved by decreasing the K_L-value and a perfect fit is obtained for the RA in tibia epiphysis with a K_L of $2.32 \cdot day^{-1}$ ($E = 0.0067$ g Ca, $L = 0.0073$ g Ca and $A = 0.0070$ g Ca$\cdot day^{-1}$). For ^{65}Zn, the fit to the RA in mandibular bone and tibia epiphysis is poorer than with larger approximations to K_L. However, the K_L-value for mandibular bone in the calculation in which E was inoperative is already quite low ($2.0 \cdot day^{-1}$) compared to those for the other bones. The fit to the curve drawn for mandibular condyle is poorer and the fit to the curve for tibia diaphysis improved by replacing the accreted fraction with a slowly exchanging compartment.

While it is not plausible that accretion of ^{85}Sr is non-existent, the true accretion rate is likely to be even less than the low value determined graphically and almost any value between these values and 0 is consistent with the data. Again, the values for E, L and A for zinc may be too low due to the ^{65}Zn SA in serum having been overestimated. Multiplying these values by 1.2 should correct for this error. None of the sums of the E- and L-values for zinc (Table 3), when corrected, exceed 75 per cent of the element in the sample and most are well below this figure. It is difficult to explain how the remainder of the zinc could come into a fraction which is unavailable for exchange when accretion is not operative in the model. Further, the SA curve for ^{65}Zn in mandibular condyle reaches its peak as it meets the serum SA curve which indicates that, if accretion is negligible, all the zinc in the condyle should be in the slowly exchangeable pool. The L-value is only 58 per cent (corrected value 70 per cent).

The straight line courses on log − log scales for the ^{85}Sr SA in serum throughout most of the experimental period and that for ^{65}Zn from 3 days may give an indication of the relative sizes of these slowly exchanging compartments. Their relative importance to the course of the serum SA may be quite different for ^{65}Zn and ^{85}Sr as only one third of the zinc and almost all of the calcium in the body is located in bone. The exchange with the zinc external to bone may dominate the serum ^{65}Zn SA course for a long time until the two have almost fully equilibrated while the ^{85}Sr

SA in serum is from the outset dominated by exchange with and accretion in bone. As the fit to the ^{85}Sr data from all four samples is improved by the inclusion of a slowly exchanging compartment and the serum ^{85}Sr SA curve behaves as it does from 0.25 day, it appears that a relatively large portion of the ^{85}Sr in bone is slowly exchanged and not accreted. The inclusion of very slowly exchanging compartments is consistent with the retention data even at later times as these compartments gain activity until they attain the serum SA, which may take a very long time, and are resorbed along with the accreted activity and returned to the exchangeable pool. The ^{65}Zn data from mandibular condyle and tibia epiphysis is not improved by the inclusion of such a compartment, which may indicate that accretion is dominating in the late retention of ^{65}Zn in these samples. The A-values in per cent for ^{65}Zn in cortical bone were approximately 60 per cent of those for ^{85}Sr and, in endochondral bone and calcifying cartilage, approximately 80 per cent of those for ^{85}Sr (corrected values 72 and 96 per cent, respectively). It may be that the ^{65}Zn data is more accurately reflecting the accretion of bone, particularly cortical bone, the ^{85}Sr data generally giving an over-estimate due to the accumulation in the slowly exchanging compartments. The observation that the hot spot to diffuse retention ratio for ^{65}Zn in cortical bone is several times that for ^{45}Ca (JOWSEY & ORVIS 1967) is consistent with this hypothesis. The retention of ^{85}Sr is expected to be very similar to that of ^{45}Ca in this respect (BAUER et coll. 1955 b). It is possible that isotopes of zinc will be used as tracer nuclides for measurement of parameters of bone anabolism in man. The short half-life and γ-emission of ^{69}Zn^{m} are well suited to this purpose (LORBER et coll. 1970).

One of the large depots of zinc which appears to affect the course of ^{65}Zn activity in serum is that in muscle (using heart muscle as representative). The same model used above was applied to the RA in heart and a perfect fit was obtained for the first four data points when $K_L = 0.92 \cdot \text{day}^{-1}$: $E = 0.0024$ mg Zn, $L = 0.0087$ mg Zn and $A = 0.0010$ mg Zn$\cdot$day^{-1}, the corrected zinc values being 20 per cent larger. According to the corrected values, the zinc which is relatively unavailable for exchange is 4.3 ppm of 17.6 ppm total and it is 'accreted' or made unavailable for exchange at the rate of 1.2 ppm$\cdot$day^{-1}. The alternative is to eliminate accretion from the model. Two methods are available. The equations derived in the Appendix may be used to directly solve for K_L, E and L from the peak and late ratios of the SA in the sample to that in serum. Using the corrected serum SA (0.83$\cdot$SA), the values obtained are $K_L = 0.69 \cdot \text{day}^{-1}$, $E = 0.0013$ mg Zn and $L = 0.0163$ mg Zn. When these values are used in the retention equation, the predicted RA exceed the observed mean RA by more than $2 \cdot \text{SE}_{\bar{x}}$. Using successive approximations to K_L to eliminate the A-fraction, the fit is still good with the following values: $K_L = 0.76 \cdot \text{day}^{-1}$, $E = 0.0024$ mg Zn and $L = 0.0117$ mg Zn. Multiplying E and L by 1.2, the total exchangeable zinc is approximately 16.9 ppm, which is very close to the total zinc in heart, 17.6 ppm. Using these values the predicted retention in the model reaches a peak at approximately 1.5 days and the predicted limit of the ratio of the heart to serum ^{65}Zn SA (K_L/

(K_L–K_s)) is 1.30 in the time period between 2 and 8 days ($K_s = 0.223 \cdot \text{day}^{-1}$) which agrees fairly well with observed ratios at 6 and 8 days of approximately 1.4 (serum SA corrected). Each of the three models is dominated by a large, slowly exchanging compartment with a turnover time ($1/K_L$) of between 1.1 and 1.4 days.

The zinc in the viscera, with the exception of pancreas, appears to be relatively rapidly exchangeable as the SA follow the serum SA (corrected) at a slightly higher level. These results agree with those of GILBERT & TAYLOR (1956). The SA in pancreas increases between 0.25 and 0.5 day even after having exceeded the serum SA level. This may indicate that zinc is in some way being actively accumulated in pancreas in a fraction which is relatively unavailable for exchange. Further data on the turnover of zinc in pancreas is presented in a subsequent article (BERGMAN & WING 1974).

Appendix

The derivation of the equation for the retention in a slowly exchanging pool and subsequent equations used to determine its size and rate of exchange.

If R_{L_t} is the amount of activity in the pool at time t, dR_{L_t}/dt is the change in the amount of activity in the pool per unit time and

$$\frac{\partial R_{L_t}}{\partial t} = K_L \cdot L \cdot \left(S_t - \frac{R_{L_t}}{L}\right), \tag{1}$$

where K_L is the fraction of the pool moving out (= in, it is assumed that steady state conditions apply) per unit time, L is the pool size (g, mg), $K_L \cdot L$ is the amount of the element moving out (= in) per unit time, and S_t and R_{L_t}/L are the specific activities in the serum and pool respectively. If it can be assumed that the serum activity decreases exponentially, that is $S_t = S_0 \cdot e^{-K_s \cdot t}$, then

$$\frac{\partial R_{L_t}}{\partial t} = K_L \cdot L \cdot S_0 \cdot e^{-K_s \cdot t} - K_L \cdot R_{L_t}. \tag{2}$$

The activity in the pool at time t is derived by first rearranging and multiplying both sides by $e^{K_L \cdot t}$:

$$\frac{\partial R_{L_t}}{\partial t} + K_L \cdot R_{L_t} = K_L \cdot L \cdot S_0 \cdot e^{-K_s \cdot t} \tag{3}$$

$$\left(\frac{\partial R_{L_t}}{\partial t} + K_L \cdot R_{L_t}\right) \cdot e^{K_L \cdot t} = K_L \cdot L \cdot S_0 \cdot e^{-(K_s - K_L) \cdot t} \tag{4}$$

$$\frac{\partial (R_{L_t} \cdot e^{K_L \cdot t})}{\partial t} = K_L \cdot L \cdot S_0 \cdot e^{-(K_s - K_L) \cdot t} \tag{5}$$

Integrating both sides with respect to t,

$$R_{L_t} \cdot e^{K_L \cdot t} - R_{L_0} = K_L \cdot L \cdot S_0 \cdot \frac{1}{K_s - K_L} \cdot (1 - e^{-(K_s - K_L) \cdot t}) \tag{6}$$

and, as $R_{L_0} = 0$, the activity in the pool at time t is

$$R_{L_t} = S_0 \cdot L \cdot \frac{K_L}{K_L - K_s} \cdot (e^{-K_s \cdot t} - e^{-K_L \cdot t}) \tag{7}$$

A special case arises when the rate constants K_s and K_L are equal. Substituting K_s for K_L in equation 1, equations 5 and 7 become

$$\frac{\partial(R_{L_t} \cdot e^{K_s \cdot t})}{\partial t} = K_s \cdot L \cdot S_0 \tag{8}$$

and

$$R_{L_t} = K_s \cdot L \cdot S_0 \cdot t \cdot e^{-K_s \cdot t} \tag{9}$$

The activity in this pool increases until it meets the serum specific activity on its downward course. After this time, the pool must begin to lose activity (equation 1), the loss of activity depending on the size of K_L in relation to K_s. If $K_L > K_s$, the specific activity in the pool approaches a constant ratio to that in serum as t becomes large

$$\frac{R_{L_t}/L}{S_t} = \frac{S_0 \cdot \frac{K_L}{K_L - K_s} \cdot (e^{-K_s \cdot t} - e^{-K_L \cdot t})}{S_0 \cdot e^{-K_s \cdot t}} = \frac{K_L}{K_L - K_s} \cdot (1 - e^{-(K_L - K_s) \cdot t}) \tag{10}$$

$$\lim_{t \to \infty} \left(\frac{R_{L_t}/L}{S_t} \right) = \frac{K_L}{K_L - K_s}. \tag{11}$$

In cases in which $K_L < K_s$ the ratio of the specific activities (equation 10) increases without limit. When $K_L = K_s$, the ratio is equal to $K_s \cdot t$ and also increases without limit. However, if $K_L \ll K_s$, the retention should decrease exponentially at later times and approach $R_{T+t} = R_T \cdot e^{-K_L \cdot t}$.

The equation for the retention in the pool becomes more complex if the serum activity cannot be represented by a single exponential over a long period of time. If the serum activity can be expressed as the sum of weighted exponentials, $S_t = S_0 \cdot \sum_{i=1}^{n} K_i \cdot e^{-K_{s_i} \cdot t}$, equation 7 becomes

$$R_{L_t} = S_0 \cdot L \cdot \sum_{i=1}^{n} K_i \cdot \frac{K_L}{K_L - K_{s_i}} \cdot (e^{-K_{s_i} \cdot t} - e^{-K_L \cdot t}). \tag{12}$$

If $K_L > K_{s_n}$, the ratio of the pool to serum specific activities will become constant at later times

$$\lim_{t \to \infty} \left(\frac{R_{L_t}/L}{S_t} \right) = \frac{K_L}{K_L - K_{s_n}}. \tag{13}$$

In many tissues it is likely that only a fraction of the element is in this slowly exchanging pool, the remainder being rapidly exchanging, accreting or inaccessable. The equation of BAUER et coll. (1955 a) for the total retention in the rapidly exchanging and accreted fractions,

$$R_t = E \cdot S_t + A \cdot \int_0^t S_t \cdot \partial t$$

can be expanded to include the slowly exchanging pool,

$$R_t = S_0 \cdot \left[E \cdot \sum_{i=1}^{n} K_i \cdot e^{-K_{s_i} \cdot t} + L \cdot \sum_{i=1}^{n} K_i \cdot \frac{K_L}{K_L - K_{s_i}} \cdot (e^{-K_{s_i} \cdot t} - e^{-K_L \cdot t}) + A \cdot \sum_{i=1}^{n} \frac{K_i}{K_{s_i}} \cdot (1 - e^{-K_{s_i} \cdot t}) \right] \tag{14}$$

The four unknown quantities E, L, K_L and A cannot be found by solving four simultaneous equations. In many cases it may be necessary to use analogue techniques. It is also possible to use successive approximations to K_L and solve three equations in A, L and E. In some cases, explicit solutions can be derived if certain assumptions can be made. If the retention of activity in the sample at later times follows a course parallel to the course of the serum activity, one might assume that little or none of the element is accreted and use a model in which only rapidly and slowly exchanging pools are present:

$$R_t = S_0 \cdot \left[E \cdot \sum_{i=1}^{n} K_i \cdot e^{-K_{s_i} \cdot t} + L \cdot \sum_{i=1}^{n} K_i \cdot \frac{K_L}{K_L - K_{s_i}} \cdot (e^{-K_{s_i} \cdot t} - e^{-K_L \cdot t}) \right] \tag{15}$$

When a stable ratio has been achieved,

$$\lim_{t \to \infty} \left(\frac{R_t}{S_t} \right) = E + L \cdot \frac{K_L}{K_L - K_{s_n}} \tag{16}$$

where R_t/S_t is the ratio of the retention in the sample to the serum specific acitvity. At the time of peak activity in the pool, t_p,

$$R_{t_p} = S_0 \cdot \left[E \cdot \sum_{i=1}^{n} K_i \cdot e^{-K_{s_i} \cdot t_p} + L \cdot \sum_{i=1}^{n} K_i \cdot \frac{K_L}{K_L - K_{s_i}} \cdot (e^{-K_{s_i} \cdot t_p} - e^{-K_L \cdot t_p}) \right] \tag{17}$$

and

$$\left(\frac{\partial R_t}{\partial t} \right)_{t_p} = S_0 \cdot \left[E \cdot \sum_{i=1}^{n} K_i \cdot (-K_{s_i}) \cdot e^{-K_{s_i} \cdot t_p} + L \cdot \sum_{i=1}^{n} K_i \cdot \frac{K_L}{K_L - K_{s_i}} \cdot (= K_{s_i} \cdot e^{-K_{s_i} \cdot t_p} + K_L \cdot e^{-K_L \cdot t}) \right] = 0 \tag{18}$$

Multiplying 17 through by K_L and adding it to 18, the $e^{-K_L \cdot t}$ term is eliminated. The resulting equation in E and L can be simplified by substituting S_{t_p} for $S_0 \cdot \sum_{i=1}^{n} K_i \cdot e^{-K_{s_i} \cdot t_p}$ and $-K_{s_p} \cdot S_{t_p}$ for $S_0 \cdot \sum_{i=1}^{n} K_i \cdot (-K_{s_i}) \cdot e^{-K_{s_i} \cdot t_p}$, the slope of the serum specific activity curve at t_p:

$$E = \left(\frac{R_{t_p}}{S_{t_p}} - L \right) \cdot \frac{K_L}{K_L - K_{s_p}}. \tag{19}$$

where R_{t_p}/S_{t_p} is the ratio of the retention in the sample to the serum specific activity at the peak. Equations 16 and 19 can be solved for K_L, E and L in terms of the total amount of the element in both pools, $T = L + E$ (if none of the element is accreted then none should be inaccessable and T can be assumed equal to the total concentration):

$$K_L = \frac{K_{s_n} \cdot K_{s_p} \cdot \lim_{t \to \infty} \left(\frac{R_t/T}{S_t} \right)}{K_{s_n} \cdot \left(1 - \frac{R_{t_p}/T}{S_{t_p}} \right) + K_{s_p} \cdot \left(\lim_{t \to \infty} \left(\frac{R_t/T}{S_t} \right) - 1 \right)} \tag{20}$$

where the ratios are those of the specific activity in the sample to that in serum at the peak and after equilibration. E and L can then be solved using either equation 16 or 19 and $L = T - E$. Note that, if the specific activity ratio at the peak is 1, that is the two specific activity curves meet at the peak, then $E = 0$, $L = T$ and

$$K_L = \frac{\lim_{t \to \infty} \left(\frac{R_t/T}{S_t} \right)}{\lim_{t \to \infty} \left(\frac{R_t/T}{S_t} \right) - 1} \cdot K_{s_n}$$

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SUMMARY

Analysis of bone retention data using 2- or 3-compartment models gives nearly equal relative accretion rates for zinc and calcium (^{85}Sr) in endochondral bone and calcifying cartilage. The rate for zinc in cortical bone is 70% of that for calcium. As the existence in cortical bone of large, slowly exchanging compartments imitating accretion is consistent with the data, the relative accretion rate for zinc may more closely reflect the relative accretion rate of cortical bone.

ZUSAMMENFASSUNG

Analysen der Knochen-Retentionsdaten unter Verwendung von 2 oder 3-Compartment-Modellen ergaben beinahe gleiche Werte für die relativen Anstiegsgeschwindigkeiten von Zink und Calcium (^{85}Sr) im endochondralen Knochen und dem verkalkendern Knorpel. Die Geschwindigkeit für Zink im cortikalen Knochen beträgt 70% der Geschwindigkeit für Calcium. Da das Vorkommen von grossen, sich langsam umsetzenden Compartments, die die Ansammlung nachahmen, mit den Daten übereinstimmt, mag die relative Ansammlungsgeschwindigkeit für Zink die relative Ansammlungsgeschwindigkeit des cortikalen Knochens eher wiedergeben.

RÉSUMÉ

L'analyse des mesures de rétention osseuse par des modèles à 2 ou 3 compartiments donne des taux relatifs d'accrétion à peu près égaux pour le zinc et pour le calcium (^{85}Sr) dans l'os enchondral et dans le cartilage en voie de calcification. Le taux pour le zinc dans l'os cortical est de 70% de celui du calcium. Comme l'existence dans l'os cortical de grands compartiments à échange lent imitant l'accrétion est compatible avec les résultats, le taux relatif d'accrétion pour le zinc pourrait refléter plus fidèlement le taux relatif d'accrétion de l'os cortical.

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