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Source of Maternal Milk Zinc for Absorption by Suckling Rats (40780)

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Zinc is essential for the proper growth and development of man and many animals (1). Zinc deficiency in young animals can cause growth retardation and impair brain development (2, 3).

For newborn animals, and for many human infants, the only source of zinc, or of any nutrient, is maternal milk. The level of zinc in milk varies among species; it is 13–17 μg/ml in rat milk (4), 3–6 μg/ml in cow milk (5), and 1.5–3 μg/ml in human milk (1, 6). The purpose of this study was to demonstrate that the zinc ingested by the suckling infant in its mother's milk must come from the maternal diet rather than her body stores.

Methods. Female Long–Evans rats about 90 days old and weighing 200 g were bred. Dams were maintained on a commercial chow diet (Purina)[1] and tap water throughout pregnancy and lactation; the chow contained 39 ppm Zn.

Dams were fed 0.5 μCi ^{65}Zn on a food pellet either immediately (Day 0) or 7 days after parturition (Groups I and II, respectively) or were injected with 0.5 μCi ^{65}Zn 3 weeks before mating (Group III). The ^{65}Zn in the dams and pups was determined daily with a Packard Armac small-animal whole-body counter (Model 446). The mean number of pups per dam in each of the three groups was 13. Dams and litters were housed in plastic tubs with cedar chips for bedding. Bedding was changed daily to minimize coprophagy by the dam.

Due to the longer time course of the experiment in Group III, dams were injected with ^{65}Zn rather than fed. Since absorption of an oral dose is rarely 100%, a larger amount of ^{65}Zn would have to be fed than was injected in order to achieve the same body burden of ^{65}Zn. Too large an amount of radioactive zinc given orally causes diarrhea and other digestive problems. If a smaller dose were given orally dams' body levels after 9 weeks (at the end of lactation) might have been difficult to detect.

Calculations were as follows:

For dams fed ^{65}Zn after delivery (on Day 0 or 7) the total ^{65}Zn dose was defined as the sum of the radioactivity in the dam and in the pups immediately after the food was consumed. Counting the food alone was not satisfactory due to differences in geometry and quenching between the rats and food pellets.

The sum of radioactivity in the dam and pups each day was expressed as a percentage of the total initial dose. When this percentage was plotted vs time on semilog paper there was a sharp initial drop followed by a linear decrease. Extrapolation of the linear portion of the curve back to zero time yielded the percentage absorption of ^{65}Zn by the dam. This is an adaptation of the method of Heth and Hoekstra (7). It was necessary to sum radioactivity in the dam and pups so that the loss of activity reflected only maternal fecal loss of ^{65}Zn; thus the y-intercept yielded the absorption value. When maternal radioactivity alone was expressed as a percentage of total dose or of initial maternal activity, the slope of the linear portion of a semilog plot reflected the rate at which zinc turnover occurred in the dam. More negative slopes indicated a more rapid turnover.

Levels of ^{65}Zn in the pups each day were expressed as a percentage of the total initial dose. Values for the sixth day and after, when the ^{65}Zn content leveled off, were av-

[1] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

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eraged, and the mean value was taken as absorption by the pups as a percentage of the total dose. This value for absorption by pups as a percentage of the total dose was divided by the percentage of the initial dose absorbed by the mother to yield the percentage absorption by the pups of the ^{65}Zn absorbed by the dam.

In the case of dams labeled before conception (Group III), increases in pup radioactivity were divided by the total amount of radioactivity in the dam immediately after delivery. The percentage values for the sixth and subsequent days were averaged to determine the percentage of the mother's body burden of ^{65}Zn at the beginning of lactation that was absorbed by the pups. For dams injected with ^{65}Zn before they were mated, the value for retention by the dam is the counts per minute in the dam on the day before delivery divided by the counts per minute in the dam immediately after injection. The ^{65}Zn level at the time of injection was used as the 100% value to calculate pups' absorption as percentage of total dose.

Results. The values for ^{65}Zn absorption for each group of dams and their litters are given in Table I.

Dams who were fed ^{65}Zn immediately after delivery (Group I) absorbed 55 ± 10% of the dose. Radioactivity in the pups rose sharply at first and then leveled off but did not fall again up to 21 days of age (as shown in Fig. 1). Values after the sixth day, when they were relatively constant, were used to calculate absorption. Pups in Group I absorbed 46 ± 4% of the ^{65}Zn that was absorbed by their dams, or 26 ± 4% of the total dose.

Dams fed labeled chow 7 days after delivery (Group II) absorbed only 12 ± 2% of the ^{65}Zn. This is significantly ($P < 0.01$) less than the value for dams given the same dose immediately after giving birth (Group I). The pups of dams in Group II absorbed 62 ± 3% of the ^{65}Zn absorbed by their mothers, or 7.4 ± 1.5% of the total dose. As a percentage of the dams' absorption, these pups absorbed a significantly higher ($P < 0.05$) percentage of the ^{65}Zn than pups in Groups I or III. As a percentage of the total dose, they absorbed significantly less ($P <$

TABLE I. ABSORPTION OF ^{65}Zn BY SUCKLING RAT PUPS AND THEIR DAMS

Group	n*	p*	Percentage total dose		Absorption by pups as percentage of dam's absorption
			Absorbed by dam	Absorbed by pups	
I. Dams labeled at parturition (Day 0)	6	12.7 ± 0.8***	55 ± 10^{b}	26 ± $4^{b,d}$	46 ± $4^{a,d}$
II. Dams labeled 7 days after parturition	3	12.8 ± 0.5	12 ± $2^{b,c}$	7.4 ± 1.5^{b}	62 ± $3^{a,b}$
III. Dams labeled before mating	5	13.4 ± 1.9	45 ± 6^{c}	4.0 ± 2.2^{d}	8.0 ± $2.1^{b,d}$

* Number of dams.
** Number of pups per litter.
*** All values are mean ± SD.
$^{a-d}$ Values in same column with same letter superscript are significantly different: $a = P < 0.05$, $b,c = P < 0.01$, $d = P < 0.001$.

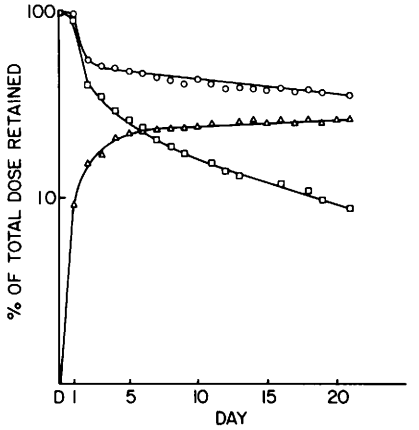

FIG. 1. Radioactivity in dams labeled immediately after delivery and in their pups (Group I). Radioactivity retained on each day is expressed as a percentage of the total initial dose. These values are the mean of six dams or six litters of pups. Open circles represent dam + pups; triangles, pups; squares, dams.

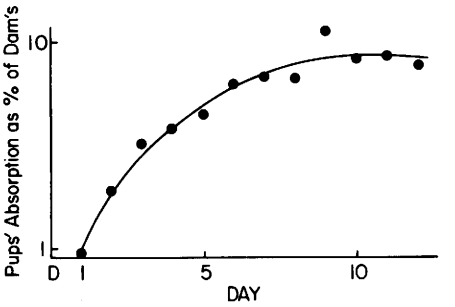

FIG. 2. Radioactivity in pups of dams labeled with ^{65}Zn 3 weeks before conception (Group III), expressed as a percentage of the total dose absorbed by the dam. Values are the mean of five litters.

0.01) than the first group. The dams and pups in Group II showed the same type of rapid decrease and increase, respectively, in radioactivity after dosing as did Group I for which data are shown graphically in Fig. 1.

Dams in Group III were injected with ^{65}Zn 3 weeks before mating (6 weeks before delivery). At the time of delivery they retained 45 ± 6% of the injected dose. This number is not comparable to the absorption values for the other two groups since it reflects retention of an injected, rather than oral, dose. The pups of dams in Group III absorbed only 4.0 ± 2.2% of the total ^{65}Zn given the dam, which is significantly ($P < 0.001$) less than absorption by pups of dams labeled at Day 0 (Group I) but not significantly different from pups of dams labeled at Day 7 (Group II). They absorbed 8.0 ± 2.1% of the ^{65}Zn which the mother contained after she delivered. This is significantly less ($P < 0.0001$) than the value for pups of dams labeled at Day 0 (Group I) and that for pups of dams labeled at Day 7 ($P < 0.01$) (Group II). The increase in radioactivity in this group of pups was not only less, but slower, than that in the other two groups, as shown in Fig. 2.

Turnover of zinc by the dams was related to the slope of the semilog plot of the dam's ^{65}Zn level vs time. The slopes of the linear portions of these curves are given in Table II. Turnover of ^{65}Zn was greatest by the dams fed labeled chow on Day 7 (Group II) after delivery, and least by those labeled with ^{65}Zn 6 weeks before delivery (Group III). All slopes differ significantly from one another.

Discussion. It is not surprising that pups nursing dams labeled with ^{65}Zn after delivery absorbed more ^{65}Zn than pups nursing dams labeled before they were conceived. Mobilization of zinc from body deposits such as bone occurs only in conditions of dietary zinc deficiency severe enough to induce bone breakdown (8, 9). These data show that when a lactating female is receiving an adequate diet, 90% or more of the zinc ingested by the suckling young originates from the dam's current dietary intake, since pups in Group III were able to absorb only 8% of the radioactivity present in the dam's body immediately after she delivered. Zinc ingested by the lactating dam passed rapidly into her milk and was soon seen in the suckling pups (Fig. 1). Pups in Groups I and II absorbed 46–62% of the ^{65}Zn ingested by their mothers.

The two groups of dams fed ^{65}Zn after delivery (Groups I and II) differed in absorption and turnover of zinc. The dams fed immediately after delivery (Group I) absorbed 55% of the zinc, compared to 12% by the dams fed on Day 7 (Group II). The dams in Group I had a slower turnover of zinc than Group II, and their pups absorbed

TABLE II. TURNOVER OF ^{65}Zn BY LACTATING RATS

Group	m
I. Dams labeled at parturition (Day 0)	$-0.034 \pm 0.002^{a,c}$
II. Dams labeled at Day 7	$-0.063 \pm 0.006^{a,b}$
III. Dams labeled before mating	$-0.012 \pm 0.003^{b,c}$

[a–c] Values with the same letter superscript are significantly different: $a = P < 0.05$, $b = P < 0.01$, $c = P$ 0.001.

a smaller percentage of what they absorbed than did pups of dams in Group II.

The high absorption and slower turnover of dams fed ^{65}Zn just after delivery (Group I) appear to indicate that much of the zinc absorbed by these dams was going to build up their own body stores. A drop in plasma zinc has been noted at the end of pregnancy even on normal diets (4), which may indicate some depletion of the dam's resources. By the seventh day after delivery (Group II), the dam's requirements had apparently lessened and absorption was only about a fifth of what it was just after parturition. A greater percentage of the zinc absorbed by the dam was diverted to the pups via the milk, and the dam's rate of zinc turnover was higher. Even though pups in Group II absorbed more ^{65}Zn as a percentage of the dam's total absorption, they absorbed less of the total dose than pups in Group I. This is probably due to the fact that zinc concentrations in colostrum are much greater than in mature milk (4, 9).

Even though the newly delivered dam used a large proportion of the zinc she absorbed for her own needs, rather than for milk, her pups still received more of the total zinc dose than the Day 7 pups, because absorption was so high just after delivery.

Turnover of zinc by dams given ^{65}Zn before mating was very low. Probably most of the zinc in these dams had equilibrated in various tissues such as bone and was not available for secretion into milk. This is reflected in the fact that only 8% of the ^{65}Zn in the dam's body after delivery showed up in the pups during the period they were nursed.

Summary. These data show that when an adequate diet is provided to the dam during lactation less than 10% of the zinc in her milk is zinc which was present in her body at parturition. It can be inferred that 90% or more of the zinc ingested by rat pups via their mothers' milk originates from the dams' current dietary sources. Dietary zinc is rapidly turned over by the lactating dam, who secretes as much as 60% of the zinc she absorbs into her milk. Zinc in body stores, which was acquired before pregnancy and lactation, is turned over much more slowly.

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