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Secretion of Dietary Strontium 90 and Calcium in Human Milk.*† (25777)

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The preferential utilization of calcium over strontium by man and many species of animals has been well studied; the concepts and magnitude of the differential behavior have been generally established(1-7). For the cow and goat under usual management, it has been shown that Sr/Ca ratio in the milk is about 0.1 of that in the diet, with values from various parts of the world falling well within the range 0.09 to 0.16(4,6,7). No direct evidence, to our knowledge, has been reported for strontium-calcium relationships between diet and human milk. It seemed desirable to obtain data on this point (a) to see if the same general relationships hold for man as for animals, since much information is being acquired under carefully controlled conditions in animals and any basis for judicious extrapolation to man might be helpful; (b) to permit estimations or predictions of Sr^{90}/Ca ratios or Sr^{90} levels in human milk from knowledge or predictions of dietary levels; and (c) to permit estimations of relationships between Sr/Ca of diet and Sr/Ca of the body in healthy individuals; this type of information has been difficult to obtain. It became possible to measure these relationships in normal healthy women by making use of Sr^{90} levels that existed in our diet during May 1959. In principle, it appears that calcium and strontium of milk are derived under normal conditions largely from calcium and strontium of the contemporary diet with a small contribution from skeletal reserves, and this most likely from regions that have been labeled recently (4,8). Thus, a study was done in which a known diet was fed to 5 women starting immediately postpartum; after a conditioning period of about 20 days, a 5-day collection of food equivalent to that eaten and a 5-day collection of all milk were obtained for analysis.

Methods. Each of the 5 subjects began 30-day participation immediately postpartum. The program was as indicated in Table I. If necessary, lactation was enhanced by pumping the breasts to assure a good volume of milk secretion and an active lactation for at least 30 days. Breast milk secreted during the last 5 days of the 30-day period was collected for analysis. a) *Dietary control.* The hospital diet was consumed during the first 5 or 6 days postpartum. On leaving the hospital the subjects went to an apartment where they were

* Actual data in this experiment refer essentially to (Sr^{90}/Ca of extracellular fluids)/(Sr^{90}/Ca of diet) during period of observation. The same $OR_{body/diet}$, however, would be expected to hold over most, if not all, of the life of the individual, as shown by previous animal experiments where short-term double tracer studies have been compared with lifetime feeding(1,2).

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TABLE I. Description of Subjects and Protocol.

Subject No.	I	II	III	IV	V
Age (yr)	20	17	22	18	22
Ht (in)	66	66	67	64.8	67.5
Wt (lb)	115	131	140	125	137
Physical condition at delivery	Good	Good	Good	Good	Good
Health during exp.	"	"	"	"	"
History of previous lactation	Grav. I Para. I	Grav. I Para. I	Grav. II Para. I	Grav. I Para. I	Grav. II Para. II
	Bottle fed by choice	Nursed by choice	Bottle fed by choice	Bottle fed by choice	Bottle fed by choice
Dates (all 1959)					
Under hospital care	May 10–15	Apr. 26–May 4	Apr. 11–20	May 8–15	Apr. 23–28
Start on controlled diet	May 15	May 4	Apr. 20	May 15	Apr. 28
Food consumption measured	May 25–June 8	May 10–26	Apr. 27–May 11	May 24–June 7	May 7–22
Food equivalent to that eaten saved for analysis, and total breast milk collected	June 4–8	May 22–26	May 7–11	June 3–7	May 18–22

served meals prepared especially for them by a trained technical group. This diet conformed to accepted standards for use during the lactation period and was approved by the physician responsible for their medical supervision. The objective was to feed a diet sufficiently constant to establish steady state with respect to calcium and strontium 90. Steps were taken to avoid sporadic variations in strontium 90 intake. Since vegetables may be rather variable in strontium 90 content, a supply of frozen and canned foods was provided in advance. This permitted all subjects to be fed from one lot throughout the period. Meat and milk were procured from day to day. The diet was not unique except that bread was excluded because preliminary data indicated possible wide fluctuations in strontium 90 concentration. It was not possible to dictate amount of food consumed each day but, although amount eaten was determined voluntarily, it was hoped that these healthy young women would maintain a rather uniform intake. From the 16th to the 30th day postpartum, each kind of food in a serving was weighed before and after the meal to determine weight consumed. Although there was considerable variation from day to day in both periods, average daily intake of the various foods did not differ markedly in the 10-day preliminary period and the 5-day collection period. This was notably the case with respect to milk products and vegetables, the 2 principal dietary sources of strontium 90. It is believed, therefore, that the subjects were reasonably in steady state in regard to diet, circulating body fluids, and accessible parts of the skeleton after the 10-day preliminary period and that conditions remained substantially the same during the 5-day collection period. b) *Collection of ingested diet and breast milk.* During the last 5 days, the collection period, a weight of each dietary item equal to amount consumed was placed in a polyethylene bottle and stored at about −15°C. All of the breast milk secreted during collection period was composited in another polyethylene bottle and stored in frozen condition. c) *Chemical analysis.* Collected food and breast milk were evaporated to dryness and ashed in a muffle at about 600°C until a white ash was obtained. The food ash was fused with sodium carbonate and the mixture dissolved in hydrochloric acid. The milk ash was dissolved directly in hydrochloric acid. The 2 quantitative estimations of calcium and strontium 90 were made by methods described by Harley and Whitney(9). d) *Expression of results.* Sufficient data are given to permit calculations on various bases. For expression of the differential movement of strontium and

TABLE II. % of Total Calcium Intake by Type of Food during 10-Day Preliminary Period and 5-Day Milk-Collection Period.*

Subject	I		II		III		IV		V	
	10 days	5 days	10 days†	5 days	10 days	5 days	10 days	5 days	10 days†	5 days
Milk products	65.4	51.5	82.4	78.7	85.1	84.4	76.4	73.0	80.8	76.7
Fruits	6.8	7.5	4.2	3.3	1.8	1.9	2.8	3.5	3.8	5.7
Fruit juice	7.4	10.3	.7	1.9	.9	1.4	3.0	3.9	.7	1.5
Vegetables	11.4	19.0	5.8	7.8	5.6	6.3	7.6	11.3	7.3	7.9
Fish and meat	2.3	3.0	1.1	1.0	1.9	1.4	1.7	1.8	2.4	1.6
Eggs	5.0	4.9	1.6	3.4	2.5	2.0	4.2	4.5	3.5	3.1
Choc. bar or candy	1.7	3.9	4.0	4.0	2.2	2.7	4.3	2.0	1.2	2.9
Peanuts			.2						.3	.5

* Calculations made from g (wet) ingested, using data in *Handbook of Biological Data*. William S. Spector, W. B. Saunders Co., Philadelphia & London, 1956.

† Preliminary period was 11 days for Subjects II and V.

calcium, the terminology of Comar(2) is used;

(Strontium-Calcium Observed Ratio):

$$OR_{milk/diet} = \frac{Sr/Ca \text{ of milk}}{Sr/Ca \text{ of diet}}.$$

Results. The calcium contribution of each major type of food was calculated by applying percentage composition as given by Spector (10) to wet weight consumed (Table II). Except in Subject I, milk products supplied about 80% of dietary calcium. These estimations are of general interest in comparing intakes during preliminary and collection periods. The figures may also be of interest in relation to population averages for calcium contributions of various diet components.

Table III presents a summary of analytical results. Total food intake was essentially the same for each subject. Food intake of Subject I differed markedly from that of the other subjects in that (a) calcium concentration of the diet was lower, (b) total calcium intake was lower, and (c) Sr^{90}/Ca ratio in total diet was higher. These differences were probably a result of the lower milk consumption of Subject I as compared with that of the others.

From about 3100 to 7200 g of milk were secreted during the 5-day period with Subject I being one of the highest producers. As expected, percentage of calcium in the milk fell in the narrow range of 0.023 to 0.028 for all subjects.

Perhaps the most important values are those for the Observed Ratio from diet to milk. OR values for Subjects II, III, IV, and V fell within a range of 0.085 to 0.132 with a mean value of 0.10; these values are in close agreement with those previously observed for goats and cows(4,6,7).

Subject I showed a markedly low value of 0.028. It is of interest to consider possible explanations for this low value. Subject I was consuming less than 1 g of calcium per day, yet secreting a relatively large amount (about 0.33 g) in her milk; it seems likely that skeletal stores, with a lower Sr^{90}/Ca ratio than the diet, were being mobilized for secretion into milk (note high Sr^{90}/Ca ratio of diet of Subject I). If this were so, then the procedure would inherently give an underestimate of true OR value. Since complete calcium and strontium balances were not run, this conclusion cannot be made unequivocally. An alternative explanation could be that comparative secretion of Sr into milk was lower for Subject I. Percentage of ingested Sr^{90} secreted/kg of milk by Subject I (0.13%, line 16) was lower by a factor of almost 3 than for Subjects II,

TABLE III. Analytical Results on Food Consumed and Milk Secreted during Collection Period by 5 Mothers.

Subject	I	II	III	IV	V
			Food eaten		
1. Total wt eaten (g)	14,780	13,800	15,390	12,380	13,220
2. " ash (g)	148.29	117.55	121.89	125.23	115.49
3. Ca as % of total wt	.03	.06	.07	.06	.05
4. g Ca in 5-day sample	4.97	8.65	10.41	7.35	7.23
5. $\mu\mu c$ Sr^{90} in 5-day sample	150.1 $\pm$4.3*	127.9 $\pm$4.0	126.6 $\pm$3.4	138.9 $\pm$ 7.8	106.0 $\pm$1.7
6. $\mu\mu c$ Sr^{90}/g Ca	30.20 $\pm$ .76	14.79 $\pm$ .09	12.16 $\pm$ .25	18.90 $\pm$ 1.06	14.66 $\pm$ .99
			Milk secreted		
7. Total wt (g)	7146	3129	4246	4069	7233
8. " vol (ml)	6930	3030	4140	3940	7000
9. " ash (g)	14.22	6.27	9.54	8.92	14.95
10. Ca as % of total wt	.023	.023	.023	.027	.028
11. $\mu\mu c$ Sr^{90}/kg of milk	.19 $\pm$ .05†	.44 $\pm$ .16	.23 $\pm$ .04	.50 $\pm$ .10	.36 $\pm$ .06
12. g Ca in 5-day sample	1.640	.709	.985	1.088	2.018
13. $\mu\mu c$ Sr^{90} in 5-day sample	1.36 $\pm$ .38	1.38 $\pm$ .51	1.01 $\pm$ .17	2.04 $\pm$ .40	2.59 $\pm$ .46
14. $\mu\mu c$ Sr^{90}/g Ca	.83 $\pm$ .23	1.95 $\pm$ .72	1.03 $\pm$ .16	1.88 $\pm$ .37	1.28 $\pm$ .22
			Relations between food eaten and milk secreted		
15. $OR_{milk/diet}$‡	.028	.132	.085	.100	.087
16. % of ingested Sr^{90} secreted/kg milk	.13	.34	.18	.36	.34
17. Sr^{90} in milk as % of ingested Sr^{90}	.91	1.08	.80	1.47	2.44
18. Ca in milk as % of ingested Ca	32.99	8.20	9.46	14.80	27.91

* Error term is one stand. dev. from the mean of duplicate analyses.

† " " " " " " due to counting.

‡ $OR_{milk/diet} = \dfrac{Sr^{90}/\text{Ca of milk}}{Sr^{90}/\text{Ca of diet}}$.

IV, and V (0.34-0.36%); however, a lowered Sr^{90} secretion (0.13 and 0.18%, respectively) could not have accounted for the difference in OR values between Subjects I and III. On the whole, mobilization of calcium stores appears to be the more likely explanation, but other possibilities cannot be ruled out. Percentage of ingested Sr^{90} secreted in total milk ranged from 0.8 to 2.4 (Table III, line 17). Subjects I, II, and III had about the same value. This again would indicate that a decreased Sr^{90} secretion into milk could not alone have accounted for the abnormally low OR value of Subject I.

Discussion. These data can be used also to estimate $OR_{body/diet}$ values. It has been shown that for the cow and goat, $OR_{milk/diet}$ averages about 0.4 times $OR_{blood/diet}$(4). If we assume the same factor for human beings, one can calculate an average $OR_{blood/diet}$ of about 0.25 for Subjects II to V; this value would be very close to the $OR_{bone/diet}$. The value of $OR_{bone/diet}$ of 0.25 is in general agreement with values estimated by other methods(3,7,11).

Some rather general statements can be made about expected levels of Sr^{90} in the infant as related to diet. It is anticipated that Sr^{90}/Ca ratio in the newborn would be about one-half Sr^{90}/Ca ratio of the plasma and therefore about 1/8 that of the diet of the mother during the period when the bone of the fetus was being formed; this arises in part from the preferential movement across the placenta of calcium as compared to strontium (5). If the newborn is to be breast fed, then the Sr^{90}/Ca ratio in the mother's milk would be about 1/10 of that in the mother's diet. On the other hand, if the newborn is to be given a formula based on cow's milk, then Sr^{90}/Ca ratio in the infant's diet would probably be close to that of the mother's diet. Unfortunately, we do not yet have any direct observation of the relationships between dietary values and Sr^{90}/Ca of the bone being formed in infants; on the basis of general knowledge, $OR_{bone/diet}$

in the infant would probably be greater than 0.25 and less than 1. These relationships can then be used, within the limitations mentioned, to estimate radiostrontium levels in young infants from knowledge of their diet or of the mother's diet during breast-feeding.

Summary. 1. In 4 out of 5 normal healthy women Sr^{90}/Ca ratio in milk ranged from 0.085 to 0.13 of diet with average 0.10. These values are essentially the same as reported for lactating animals. The value for the other subject was 0.028, and it is considered most likely that this was a result of relatively low calcium intake (about 1 g/day), which probably led to marked negative calcium balance and thus invalidated the procedure. 2. If we assume relationship between Sr/Ca ratio in blood and milk is the same for the human as for cow and goat, it can be calculated that Sr/Ca ratio in these subjects would be about 0.25 that in diet consumed. This value is in agreement with other values obtained for man by methods based on other assumptions.

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Effect of Dilantin Sodium on Growth of Human Fibroblast-Like Cell Cultures.* (25778)

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Fibrous hyperplasia of the gingiva occurring as an untoward reaction following systemic administration of sodium diphenylhydantoinate (Dilantin Sodium) in treatment of epilepsy is a well recognized clinical phenomenon. Microscopic examination of this hyperplastic tissue characteristically shows increased fibroblastic activity with pronounced formation of new collagen fibers. Shapiro(1) has reported that administration of Dilantin to non-epileptic patients increased rate of healing of experimentally induced gingival wounds through increased connective tissue activity as well as accelerated epithelialization. In support of this clinical finding, Shafer *et al.*(2) noted that administration of Dilantin Sodium to rats produced a dramatic increase in tensile strength of healing skin wounds. They suggested that this was probably a function of increased collagenization, the same reaction which occurs in the gingival hyperplasia of patients receiving this drug. In an attempt to learn more of the mechanism for both the gingival hyperplasia and the apparent stimulation of wound healing, a study has been carried out testing the effect of Dilantin Sodium on rate of growth of human fibroblast-like cells in cell culture.

Materials and methods. The strain of fibroblast-like cells (designated as E.D.) used in these studies was originally isolated from adult human gingiva by Dr. Donald E. Flieder, St. Louis Univ. School of Dentistry and was courteously supplied by him. At the time of ini-

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