Strontium–Calcium Discrimination During Placental Transfer and Fetal Uptake in Rats: Effect of Gestation Duration[1] (37317)

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In the physiological processes of intestinal absorption and renal excretion as well as placental transport, fetal uptake, and milk secretion, discrimination against strontium in favor of calcium has been shown to occur (1).

Diet composition in its effect on intestinal absorption of calcium and strontium and strontium–calcium discrimination during absorption has been extensively investigated. Dietary lactose (2, 3) amino acids (3–5), phosphorus (6), and magnesium (7) have been reported to modify intestinal absorption of strontium and calcium and/or strontium–calcium discrimination. Similarly the amino acid profile of dietary protein (protein nutritive value) has been shown to affect these criteria (8).

Placental transport and fetal uptake of Ca and Sr and of ^{45}Ca and ^{90}Sr have been studied in several species. Comar (9) reported that Ca uptake by rat fetuses increased from 0.018 mg/fetus/day at Day 14 to 12.3 mg/fetus/day at Day 22, and that the rate of increase in Ca uptake was relatively constant to Day 19. Pregnant rats given ^{45}Ca orally each day during gestation evidenced fetal content of 4.04% of the administered dose (10). During gestation sows on normal (0.7%) dietary Ca transferred more total Ca and ^{45}Ca to fetuses and deposited more ^{45}Ca in the maternal carcass than sows receiving limited (0.3%) dietary calcium (11). The same work revealed greater Ca and ^{45}Ca concentration in the fetuses than in the dam. In similar work from the same laboratory (12) litters from sows dosed orally or iv with ^{45}Ca at gestation periods of 35–114 days contained 0.9–21.3% of absorbed ^{45}Ca at 168 hr after administration of the dose. In the same animals less than 1% of the absorbed dose was retained in combined placenta and fluids. In ewes given a single iv dose of ^{45}Ca in one trimester of pregnancy it was reported (13) that fetal tissues generally paralleled maternal tissues in ^{45}Ca accretion. The total fetuses were found to contain approximately 0.4, 4, and 35 g of calcium at trimester 1, 2, and 3, respectively, and approximately 2, 10, and 20% of the administered dose of ^{45}Ca. Strontium uptake in fetuses of dogs chronically exposed to ^{90}Sr at equilibrium with ^{90}Y has been measured (14). The observed placental transfer to the litter was 1.4 ± 0.3% of the administered dose and was 4.9 ± 0.8% of the activity retained in the maternal skeleton. Oral administration of ^{90}Sr to cows throughout pregnancy resulted in passage through the placenta of 0.38 to 1.73% of the administered dose (15); the ratio of ^{90}Sr:Ca during passage from mother to fetus was observed to vary from 0.007 to 0.16. Administration by a single iv dose of ^{45}Ca and ^{90}Sr at equilibrium with ^{90}Y to female rats 12 days before mating showed that passage of Sr to the fetuses was impeded in favor of Ca (16). The placental discrimination against Sr was verified by the existence of a fetal ^{90}Sr:^{45}Ca (observed ratio, OR[2]) of 0.54 ± 0.07. The

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[2] The strontium–calcium observed ratio (OR) is defined in the following manner (1): $OR_{sample-precursor}$ = Sr/Ca of sample/Sr/Ca of precursor. This notation, $OR_{fetus-dose}$, reduces to fetal Sr (% of dose)/fetal Ca (% of dose) when Sr and Ca of the sample (*i.e.* fetal) are expressed as percentages of administered dose and Sr/Ca of the precursor (*i.e.*, the dose) is unity.

same work reported OR in maternal skeleton, at birth, of 1.11, 0.97 and 1.06 in cranial bone, spine, and femur, respectively.

The facts that diet composition affects intestinal Ca and Sr absorption and that Sr:Ca discrimination occurs in passage of these substances through membranes are well established. However, to our knowledge, no experimental work has been presented in which were observed the effect of dietary composition upon placental transport of Ca and Sr or Sr:Ca discrimination during gestation. And although a decrease in OR was reported during early postnatal growth (17), no observations of OR of fetal tissue during gestation have been reported.

The purpose of the work reported here was to observe longitudinally throughout the final trimester of gestation the effect of widely differing casein content of semisynthetic diets upon the transfer of ^{45}Ca and ^{89}Sr across the placentas of laboratory rats. The dietary percentage of casein was found to affect significantly neither the placental transport of the radioisotopes nor the discrimination against ^{89}Sr transport. However, systematic variation was observed in the ^{89}Sr:^{45}Ca ratio of the fetuses during gestation.

Materials and Methods. Animals and treatments. Twenty-eight nulliparous female rats of the Wistar strain, housed in an animal room maintained at 24 ± 2°, were employed in this work. At the initiation of the work the animals, averaging 242.5 g in body weight and 105 days of age, were randomly assigned to two outcome groups of 14 animals each and given *ad libitum* tap water and either a 10 or 22% protein diet (Table I). The female rats, in groups of two or three, were housed in screen-bottom galvanized steel cages with a male rat of the same species. When daily vaginal smears were found positive for viable sperm, the female rats were placed in individual screen-bottom cages and that day was counted as Day one of gestation.

At intervals from Days 13 to 21 of gestation the animals were administered via the tail vein and at a dosing rate of 0.25 ml/100 g animal a dosing solution containing (on the day of assay) 0.025 mCi of ^{45}Ca and 0.020 mCi of ^{89}Sr/ml.[3] The animals were then placed in metabolism cages for the quantitative separate collection of feces and urine. Exactly 24 hr after dosing, the animals were killed with diethyl ether and the GI tract and gravid uterus were excised. The fetuses were removed from the uterus, rinsed with distilled water, blotted with filter paper, and weighed. The uterine wall and placental structures and fluids were combined with fetal washings and designated "placenta." The GI tract contents were removed and the tract was included with the remainder of the maternal carcass.

Preparation and assay of samples. The samples analyzed were: 24-hr urine and feces, GI tract contents, maternal carcass, fetuses and "placentas." They were dried to constant weight at 105° and weighed, ashed in a

TABLE I. Composition (%) of Experimental Diets.

Ingredients	Protein (%)	
	10	22
Crude casein[a]	11.25	24.75
Corn oil	5.00	5.00
Glucose monohydrate	40.55	27.05
Corn starch	35.00	35.00
Vitamin mix[b]	2.20	2.20
Mineral mix[c]	4.00	4.00
Cellulose	2.00	2.00

[a] Supplied by Nutritional Biochemicals Corp., Cleveland, OH and containing 88.9% protein (N × 6.25).

[b] Vitamin diet fortification mixture in dextrose (Nutritional Biochemicals Corp., Cleveland, OH) containing (mg/g of mixture): vitamin A concentrate (crystalline retinyl acetate, 200,000 U/g), 4.5; vitamin D concentrate (crystalline ergo calciferol 400,000 U/g), 0.25; All *rac.*-α-tocopherol, 5.0; ascorbic acid, 45.0; myo-inositol, 5.0; choline chloride, 75.0; menadione, 2.25; *p*-aminobenzoic acid, 5.0; nicotinic acid, 4.5; riboflavin, 1.0; pyridoxine·HCl, 1.0; thiamin·HCl, 1.0; calcium pantothenate, 3.0; and (μg/g of mixture) biotin, 20.0; folic acid, 90.0; and cyanocobalamin, 1.35.

[c] Jones and Foster (18).

[3] $^{45}CaCl_2$ solution (sp act 15.1 mCi/mg Ca) obtained from New England Nuclear Corp., Boston, MA. $^{89}SrCl_2$ solution (carrier-free) obtained from Union Carbide Corp., Oak Ridge, TN.

muffle furnace at 550°, and the ash was extracted with 2 *N* HCl and made to volume. Radioactivity of the samples was counted on a liquid scintillation spectrometer,[4] using standard techniques for double-labeled samples.

Results and Discussion. The group means for all response criteria for the 10 and 22% protein diets were compared using the conventional *t* test (19) and were found to not differ significantly (*i.e.*, $p > 0.05$ in every case). Therefore, the responses for both protein levels were pooled.

The percentages of injected dose of ^{45}Ca and ^{89}Sr appearing in maternal carcass, urine, feces and GI tract contents appear in Table II along with the ratio of ^{89}Sr:^{45}Ca (OR). The percentages of injected dose of the isotopes appearing in fetuses and "placentas" (both as the quantity in total tissue and per gram of tissue) appear in Table III along with the OR. It should be noted that one maternal carcass sample, 10 feces samples and 2 fetus samples were lost in preparation for analyses. A defective controller of the muffle furnace caused these samples to be seriously overheated.

The greater portion of labeled Sr and Ca of the administered dose was found in the maternal carcass (Table II)—with the quantities present decreasing as gestation progressed. Regression analyses of maternal carcass ^{89}Sr and ^{45}Ca and the ^{89}Sr:^{45}Ca ratio as functions of duration of gestation yielded the following results: (a) Maternal carcass content of ^{89}Sr and ^{45}Ca during 13–21 days of gestation were adequately described by linear regression lines whose slopes were −2.78 and −4.33, respectively. The regression coefficients, each significantly different from zero but not different from one another, explained significant portions of the variance due to gestation time ($p < 0.05$ and < 0.001 for ^{89}Sr and ^{45}Ca, respectively). This finding of decreased maternal ^{45}Ca as gestation progressed is at variance with the lack of effect of stage of gestation on maternal ^{45}Ca reported by Hoskins and Hansard (13) in the ewe. (b) the ^{89}Sr:^{45}Ca ratio of maternal carcass, generally less than unity, increased significantly during 13–21 days of gestation. This increase was adequately described by a linear regression whose slope, 0.024, explained a significant portion ($p < 0.01$) of the variance due to gestation time. Thus during gestation ^{89}Sr was increasingly selectively retained relative to ^{45}Ca.

Urinary excretion of ^{89}Sr at all observed intervals of gestation exceeded that of ^{45}Ca causing the OR of the urine to be significantly greater than unity, indicating that ^{89}Sr was selectively excreted relative to ^{45}Ca. This observation is in accord with previously reported summary data given by Comar (1).

Fecal excretion and the contents of the GI tract were notable in containing much greater percentages of the administered dose of ^{89}Sr than of the ^{45}Ca. The resulting OR was significantly greater than unity at all periods tested, indicating that ^{89}Sr was selectively excreted relative to ^{45}Ca—a finding also in agreement with previous summary data given by Comar (1).

The percentage of dose of ^{89}Sr and ^{45}Ca taken up by fetuses and "placentas" with varying duration of gestation appear in Table III. The fetal ^{89}Sr and ^{45}Ca increased in the typical logarithmic fashion, but the placental isotope content remained relatively constant. The curves of fetal uptake are similar in shape to those found for fetal growth by other workers (12, 20) and the percentage of administered ^{45}Ca found in the fetuses near term compares favorably with the 21.3% of administered ^{45}Ca found in fetal pigs at 114 days of gestation (12). The decreased fetal uptake of ^{89}Sr and ^{45}Ca at 18 and 21 days gestation was due to the decreased rate of fetal growth in late gestation. This effect was evident in calcium uptake by fetal rats (9) and was also noted in fetal pigs late in the third trimester of pregnancy (12). The percentage of ^{45}Ca found in "placentas" compares favorably with the 0.142% of administered ^{45}Ca found in placenta and fluids in pigs at 114 days of gestation (12).

Figure 1 presents the percentage of dose of the two isotopes on the ordinate and the fresh weight of fetuses on the abscissa. From

[4] Model No. 3375, Packard Instrument Co., Inc., Downers Grove, IL.

TABLE II. Deposition of a Single iv Dose of ^{89}Sr and ^{45}Ca in Maternal Carcass and Maternal Excretion During the Subsequent 24 hr as a Function of Duration of Gestation in the Rat.

Disposition of injected dose	Day of gestation							
	13	15	16	17	18	19	20	21
Maternal carcass	(2)[a]	(2)	(4)	(4)	(4)	(6)	(3)	(2)
^{89}Sr (% of dose)	83.8 ± 5.8[b]	76.3 ± 0.2	63.3 ± 9.3	65.9 ± 5.3	75.6 ± 1.7	67.9 ± 5.0	50.5 ± 8.2	58.2 ± 4.7
^{45}Ca (% of dose)	91.1 ± 5.1	87.5 ± 0.4	76.7 ± 7.9	74.3 ± 6.8	82.8 ± 4.6	67.0 ± 2.2	52.2 ± 4.1	63.1 ± 0.2
$^{89}Sr/^{45}Ca$	0.92 ± 0.01	0.87 ± 0.01	0.81 ± 0.04	0.89 ± 0.03	0.92 ± 0.05	1.01 ± 0.05	0.95 ± 0.08	0.92 ± 0.07
Urinary excretion	(2)	(2)	(4)	(4)	(4)	(6)	(4)	(2)
^{89}Sr (% of dose)	3.47 ± 0.16	2.85 ± 1.08	7.18 ± 2.05	4.18 ± 0.71	3.85 ± 0.45	1.81 ± 0.43	2.16 ± 0.59	2.61 ± 1.09
^{45}Ca (% of dose)	1.91 ± 1.14	0.46 ± 0.03	1.48 ± 0.57	0.95 ± 0.08	1.57 ± 0.53	0.73 ± 0.16	0.78 ± 0.20	0.52 ± 0.13
$^{89}Sr/^{45}Ca$	2.66 ± 2.01	6.29 ± 2.67	6.51 ± 1.52	5.24 ± 1.04	5.56 ± 3.69	3.04 ± 0.71	2.81 ± 0.58	4.79 ± 0.90
Fecal excretion	(1)	(1)	(2)	(4)	(3)	(2)	(3)	(2)
^{89}Sr (% of dose)	0.2	0.4	1.8 ± 1.7	1.3 ± 0.7	1.4 ± 0.8	2.5 ± 2.3	2.4 ± 2.2	5.2 ± 3.7
^{45}Ca (% of dose)	0.2	0.2	0.9 ± 0.8	0.8 ± 0.4	0.9 ± 0.5	0.9 ± 0.7	1.0 ± 0.8	1.6 ± 0.7
$^{89}Sr/^{45}Ca$	1.4	1.7	1.6 ± 0.6	1.2 ± 0.2	1.5 ± 0.1	2.1 ± 0.8	1.5 ± 0.5	2.8 ± 1.0
Contained in GI tract	(2)	(2)	(4)	(4)	(4)	(6)	(4)	(2)
^{89}Sr (% of dose)	10.2 ± 0.8	17.6 ± 9.2	21.9 ± 7.9	11.3 ± .6	10.4 ± 2.7	12.6 ± 3.6	13.2 ± 4.0	6.9 ± 3.2
^{45}Ca (% of dose)	4.4 ± 0.3	8.8 ± 8.0	9.1 ± 5.2	3.1 ± .8	2.2 ± 0.8	5.7 ± 2.3	6.6 ± 2.5	0.5 ± 0.2
$^{89}Sr/^{45}Ca$	2.3 ± 0.0	6.1 ± 4.5	2.8 ± 1.0	4.7 ± 1.4	5.9 ± 1.6	6.7 ± 4.8	2.2 ± 0.2	23.1 ± 17.7

[a] Numbers enclosed in parentheses are number of observations.

[b] Mean ± standard error.

TABLE III. Uptake of a Single iv Dose of ^{89}Sr and ^{45}Ca by Placental Structures and Fetuses During the Subsequent 24 hr as a Function of Duration of Gestation in the Rat.

	Day of gestation							
	13	15	16	17	18	19	20	21
Placenta	(2)[a]	(2)	(4)	(4)	(4)	(6)	(4)	(2)
Wt (g)	8.6 ± 2.4[b]	5.4 ± 3.6	13.3 ± 2.8	18.6 ± 2.2	15.8 ± 5.8	24.5 ± 1.9	19.0 ± 2.8	7.3 ± 2.5
^{89}Sr (% of dose)	0.36 ± 0.04	0.19 ± 0.15	0.48 ± 0.15	0.62 ± 0.08	0.55 ± 0.20	0.86 ± 0.06	0.59 ± 0.03	0.27 ± 0.12
^{45}Ca (% of dose)	0.41 ± 0.01	0.26 ± 0.18	0.70 ± 0.19	0.79 ± 0.15	0.62 ± 0.18	0.84 ± 0.06	0.52 ± 0.09	0.23 ± 0.05
^{89}Sr (% dose/g)	0.088 ± 0.008	0.030 ± 0.008	0.033 ± 0.006	0.033 ± 0.001	0.036 ± 0.002	0.035 ± 0.002	0.032 ± 0.003	0.035 ± 0.005
^{45}Ca (% dose/g)	0.053 ± 0.015	0.045 ± 0.005	0.050 ± 0.006	0.042 ± 0.005	0.043 ± 0.004	0.035 ± 0.003	0.027 ± 0.001	0.033 ± 0.005
$^{89}Sr/^{45}Ca$	0.87 ± 0.10	0.65 ± 0.11	0.64 ± 0.08	0.82 ± 0.11	0.85 ± 0.08	1.02 ± 0.06	1.19 ± 0.13	1.12 ± 0.31
Fetuses	(2)	(2)	(4)	(4)	(4)	(5)	(3)	(2)
Wt (g)	1.6 ± 0.6	2.0 ± 0.2	5.2 ± 1.3	14.1 ± 5.6	13.0 ± 6.5	26.4 ± 2.2	36.0 ± 3.3	23.3 ± 0.10
^{89}Sr (% of dose)	0.11 ± 0.02	0.07 ± 0.02	0.24 ± 0.05	5.35 ± 4.05	4.66 ± 2.67	15.54 ± 1.16	18.97 ± 0.90	12.91 ± 2.76
^{45}Ca (% of dose)	0.14 ± 0.02	0.09 ± 0.02	0.49 ± 0.07	9.29 ± 6.78	8.30 ± 4.54	25.07 ± 2.13	29.41 ± 1.66	20.20 ± 7.79
^{89}Sr (% dose/g)	0.071 ± 0.016	0.037 ± 0.004	0.052 ± 0.006	0.260 ± 0.108	0.362 ± 0.043	0.562 ± 0.034	0.581 ± 0.049	0.554 ± 0.121
^{45}Ca (% dose/g)	0.096 ± 0.027	0.045 ± 0.004	0.115 ± 0.030	0.471 ± 0.174	0.705 ± 0.114	0.900 ± 0.029	0.904 ± 0.101	0.868 ± 0.338
$^{89}Sr/^{45}Ca$	0.75 ± 0.04	0.83 ± 0.01	0.49 ± 0.05	0.53 ± 0.02	0.53 ± 0.04	0.63 ± 0.04	0.65 ± 0.03	0.69 ± 0.13

[a] Numbers enclosed in parentheses are number of observations.

[b] Mean ± standard error.

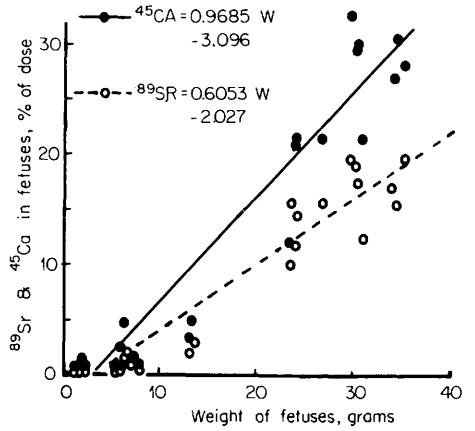

FIG. 1. Relationships of fetal ^{89}Sr and ^{45}Ca to fetal weight.

these data it is clear that fetal uptake of each isotope is a linear function of attained fetal weight. It is also clear that the uptake of ^{45}Ca is greater than ^{89}Sr. Regression lines were fitted to the data and their equations appear in the figure. The slopes of the regression lines were found to differ significantly ($p < 0.001$). Although the curves do not pass through the origin, it is felt that the deviation is not great enough to detract from the validity of data interpretation.

The OR of fetuses versus duration of gestation is plotted in Fig. 2. The similar plot for the placental structures is shown in Fig. 3. The general tendency for the ratio to attain minimal values for fetuses at 16 to 18 days of gestation is evident in Fig. 2. At values of gestation in excess of 18 days, the

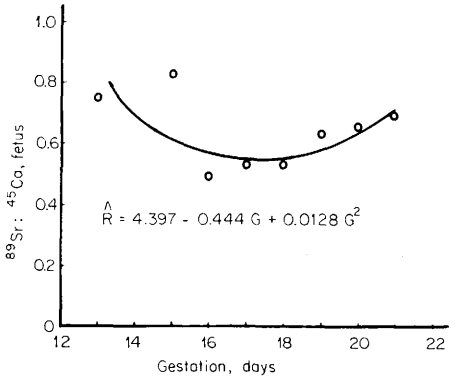

FIG. 2. Relationships of fetal ^{89}Sr:^{45}Ca ratio to duration of gestation in the rat.

fetal OR tends toward higher values, and at duration less than 16 days the tendency is also toward higher values. The value of the ratio, however, ranges within the limits of approximately 0.5 and 0.8. This range of values may be compared with the value of 0.54 in the newborn rat, previously reported (16). The OR for placentas, on the other hand, tended to attain minimal values at 15 and 16 days of gestation (Fig. 3) and at greater values of time to exhibit rapidly increasing values—so that at values of 20 days or longer the ratio attained values in excess of unity. That is, ^{89}Sr was preferentially retained by the placenta in late gestation. Since the OR of both fetuses and placentas appeared to change in a regular fashion with duration of gestation, quadratic regression

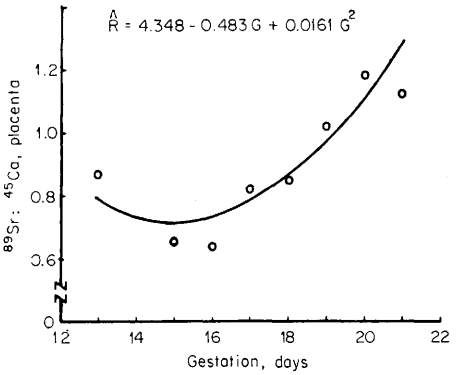

FIG. 3. Relationship of placental ^{89}Sr:^{45}Ca ratio of duration of gestation in the rat.

lines were fitted to the relationships (19). These regression coefficients were found to explain significant proportions of the variance due to treatment and to explain significantly greater portions of variance due to treatment than did respective linear regression. The equations of the regression lines are shown in Figs. 2 and 3. The relationship of the discrimination occurring in net passage of the isotopes from maternal carcass to fetuses (OR fetus–maternal, or OR fetus/OR maternal carcass) with the duration of gestation T was also found to be adequately described by the quadratic regression equation: $OR_{fetus-maternal} = 5.77 - 0.585T + 0.016T^2$.

Summary. The purposes of this study were: (a) to quantitate longitudinally the uptake of ^{89}Sr and ^{45}Ca and the strontium–calcium discrimination of placental structures and fetuses during the final third of the rat gestation period. And, (b) to determine whether dietary protein percentage affects these responses. Twenty-eight female rats, receiving 10 or 22% casein diets, were given iv doses of ^{89}Sr and ^{45}Ca proportional to body weight on either day 13, 15, 16, 17, 18, 19, 20 or 21 of gestation. One day after dosing ^{89}Sr and ^{45}Ca content of fetuses, placentas, maternal carcass and GI tract contents were determined along with 24-hr urine and feces content. Dietary protein percentages had no significant effect on any experimental criterion. Pooled data for both diets showed uptake by placental structures of 0.36 and 0.27% of the ^{89}Sr dose and 0.41 and 0.23% of the ^{45}Ca dose at 13 and 21 days gestation, respectively. Parallel values for fetal uptake were 0.11 and 12.91% of the ^{89}Sr dose and 0.14 and 20.20% of the ^{45}Ca dose, respectively. The strontium–calcium discrimination [*i.e.*, ^{89}Sr:^{45}Ca ratio, or observed ratio (OR)] evident in placental structures, total fetuses and between fetuses and maternal carcasses changed curvilinearly with days duration of gestation (T) according to these regression equations: $OR_P = 4.348 - 0.483T + 0.016T^2$, $OR_F = 4.397 - 0.444T + 0.013T^2$, and $OR_{F/M} = 5.77 - 0.585T + 0.016T^2$; where OR_P, OR_F, and OR_M are the ^{89}Sr:^{45}Ca ratios of placental structures, fetuses, and maternal carcass, respectively, and $OR_{F/M}$ is OR_F/OR_M.

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