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Age-Related Changes in Strontium to Calcium Ratios in Rat Tissues

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ABSTRACT

The Sr/Ca ratios in plasma, urine, bone, and soft tissues for various ages after weaning in male and female rats were determined to examine the effects of aging on the discrimination between strontium (Sr) and calcium (Ca) under physiological conditions. Age-related changes in the Sr/Ca ratios were similar in all tissues; the Sr/Ca ratios decreased rapidly until about 25-wk-old and then slowly, from that period on, reaching much lower values than in the diet. When the logarithm of the Sr/Ca ratio in each tissue was plotted against the logarithm of age, a linear relationship was observed with statistically significant ($p < 0.05$) regression lines. The higher levels of Sr/Ca ratios in all tissues of the younger rats could be explained by the high efficiency of Sr absorption by the small intestine early in life. Parameters for the equations between age and Sr/Ca ratio differed with tissues, suggesting the existence of specific discrimination mechanisms in each tissue.

Index Entries: Strontium; calcium; discrimination; aging, effects of; strontium/calcium ratio; rat tissues.

INTRODUCTION

Among the protective mechanisms from harmful elements, discrimination between essential and nonessential elements is considered to play an essential role in the body. The chemical properties of strontium (Sr) are very similar to those of calcium (Ca) (*1*) and, indeed, the metabolism

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and distribution of Sr in the body closely resemble those of Ca. However, discrimination against Sr in favor of Ca has been shown to occur during the physiological processes of intestinal absorption, renal excretion, fetal uptake, and milk secretion (2–4). Comar and Wasserman (2) suggested that whenever there is a metabolically-controlled passage of ions across a membrane, Ca is transported more effectively than Sr. For a better understanding of the discrimination in living animals, it may be useful to investigate Sr/Ca ratios in the tissues since Sr/Ca ratios reflect consequences of the discrimination that is produced by one or more physiological processes, such as intestinal absorption, renal excretion, or uptake by each tissue. The discrimination between Ca and Sr could be affected by physiological and nutritional factors. Age is one of the important physiological factors. However, age-related changes in the comparative distribution of Ca and Sr in the body have not yet been studied.

The recent development of inductively coupled argon plasma-atomic emission spectrometry (ICP) has made it possible to determine the trace concentrations of Sr simultaneously with Ca in biological materials. Therefore, it is possible to obtain data on the comparative distribution of Ca and Sr under physiological conditions without interfering with exogenous elements.

In the present study, we examine age-related changes in the Sr/Ca ratio in plasma, urine, bone, and soft tissues of rats after weaning and discuss the effects of aging on discrimination between Ca and Sr. We also present the Sr/Ca ratios in tissues of rats over 2-yr-old, studied to investigate the effects of senility.

MATERIALS AND METHODS

Thirty-seven male and 43 female 4-wk-old Wistar rats were purchased from Clea Japan Co., Tokyo and kept in stainless steel cages with free access to distilled water and a standard laboratory chow (CE-2, Clea Japan Co.) containing 1093 mg Ca/100 g and 2.662 mg Sr/100 g (Sr/Ca $\times$ 10^3 = 2.43). All animals were fed on the same diet throughout the experiment. Three to five animals of each sex at the following ages were individually transferred to glass metabolism cages (Sugiyama-gen Co., Tokyo) cleaned with 10% HNO_3 (5, 9, 13, 17, 21, 25, 37, 50, and 96 (three females only) weeks of age). The urine for each animal was collected over a 24-h period, and then the animals were sacrificed by exsanguination under pentobarbital anesthesia. The following organs and tissues were removed: liver, kidney, pancreas, spleen, lungs, heart, muscle, duodenum, and femur. In addition, other male Wistar rats at 6-wk-old were maintained on the standard laboratory chow. At the ages of 110, 114, 118, and 134 wk, four or five animals were treated in a similar manner to obtain data for old rats.

The urine specimens were diluted appropriately with distilled water. Heparinized blood was centrifuged at 2000*g* for 10 min. Portions (0.5 mL)

of the urine specimens and plasma were each digested with 1 mL concentrated acid mixture ($HNO_3/HClO_4$ = 5:1) for the determination of Ca and Sr concentrations. Portions (0.2–0.3 g) of the tissues and the right femur were also digested. The wet-digested solution was diluted appropriately with distilled water, and Ca and Sr concentrations were analyzed by ICP (JY48PVH, Seiko Instruments & Electronics Ltd., Tokyo). Statistical analyses (containing calculation of correlation coefficients and regression lines, regression analyses, and tests of similarities in the regression lines) were conducted using the Biochemical Computer Program-P (*5*).

RESULTS

Age-related changes in the Ca and Sr concentrations in the duodenum, plasma, and femur are shown in Fig. 1. In both male and female rats, the changes in Ca and Sr concentrations in the duodenum were similar; both Ca and Sr concentrations tended to be higher in young rats than adult animals. In the rats, aged from 110-wk-old, large differences among individuals were observed. Plasma Ca concentrations changed only slightly with age, whereas plasma Sr concentrations decreased with age in male and female rats. In the femur, Ca and Sr concentrations increased until 25 wk of age, and then decreased slowly after this time. In the soft tissues, the changes in Ca concentration showed different patterns from Sr (Fig. 2). In all tissues except the thyroid and lung, Sr concentrations tended to decrease with age in female rats. The patterns of changes in Ca concentration differed with tissues or sex. Urinary Ca excretion tended to increase with age in female rats, whereas urinary Ca in male rats and Sr in both sexes did not show any consistency (Fig. 3).

Plasma, urine, and all tissues showed an age-related decrease in the Sr/Ca ratio (Figs. 4–6), with a rapid decrease until about 25 wk of age and then a slow decrease in both male and female rats after this time. No definitive changes in the Sr/Ca ratio were observed in the rats over 2-yr-old. In order to evaluate the patterns of changes in Sr/Ca ratios, we examined the relationship between age and Sr/Ca ratio in each tissue and analyzed the results mathematically. When the logarithms of the Sr/Ca ratio in plasma, urine, and the tissues of each sex were each plotted against the logarithm of age, straight lines were observed in each case. Therefore, the relationship between age and the Sr/Ca ratio in each tissue could be expressed by the following equation:

$$\log (Sr_T/Ca_T \times 10^3) = \alpha + \beta \log A$$
$$Sr_T/Ca_T \times 10^3 = \alpha' A^{\beta} \quad (5 \leq A \leq 134)$$

where Sr_T and Ca_T = Sr and Ca concentrations in plasma, urine or each tissue; A = age (weeks); α and β = specific constants to plasma, urine, or each tissue; and $\alpha' = 10^{\alpha}$. The parameters of the resulting regression lines have been shown in the Table 1. The regression analyses indicated that all regression lines were statistically significant ($p < 0.05$). All β

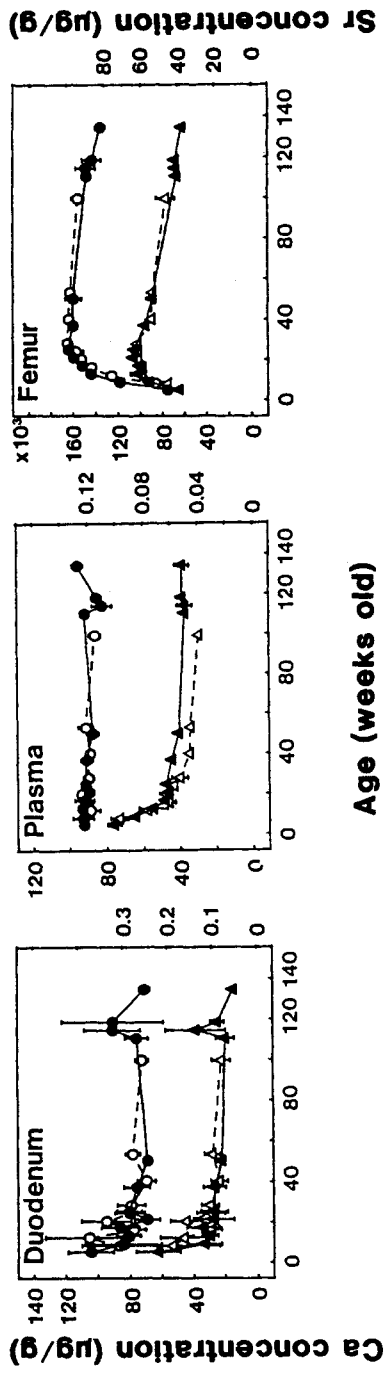

Fig. 1. Age-associated changes in Ca and Sr concentrations in rat duodenum, plasma, and femur. Each data point represents mean ±SD for 3–5 rats. ●: Ca, male; ○: Ca, female; ▲: Sr, male; △: Sr, female.

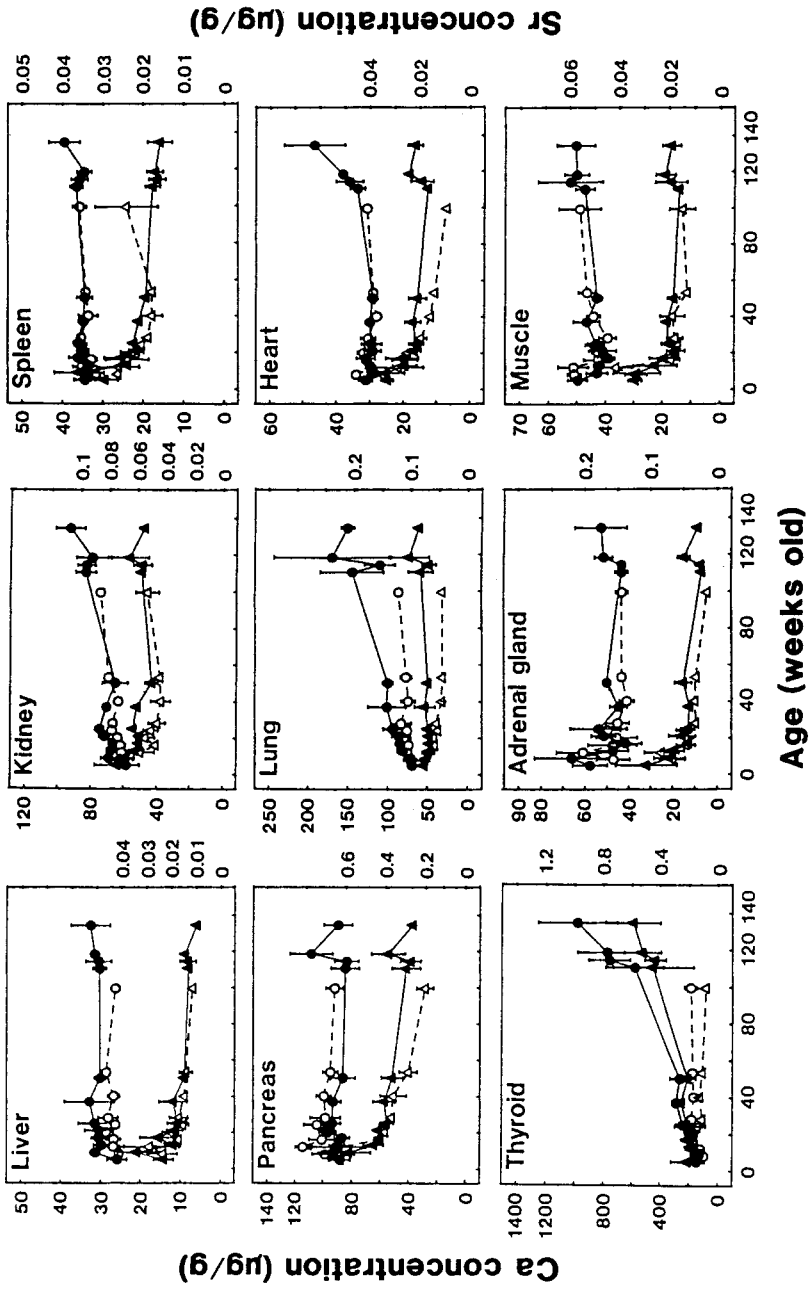

Fig. 2. Age-associated changes in Ca and Sr concentrations in rat soft tissues. *See* legend to Fig. 1.

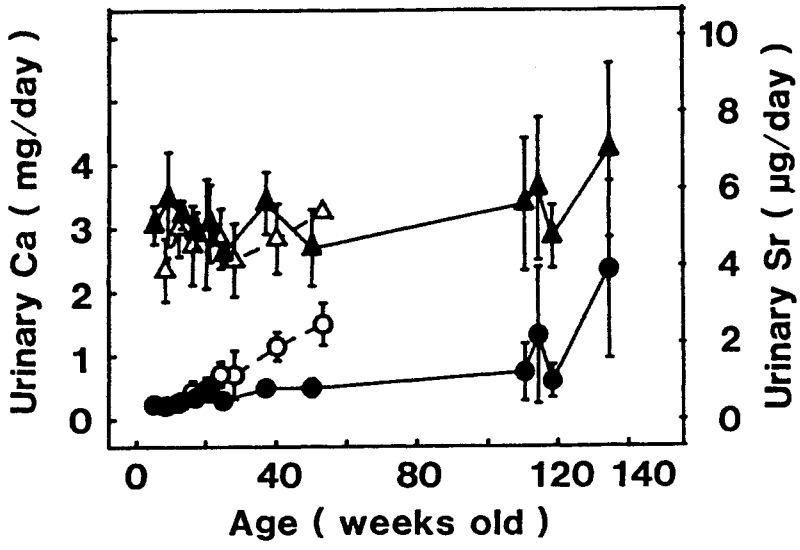

Fig. 3. Age-associated changes in Ca and Sr excretion in rat urine. *See* legend to Fig. 1.

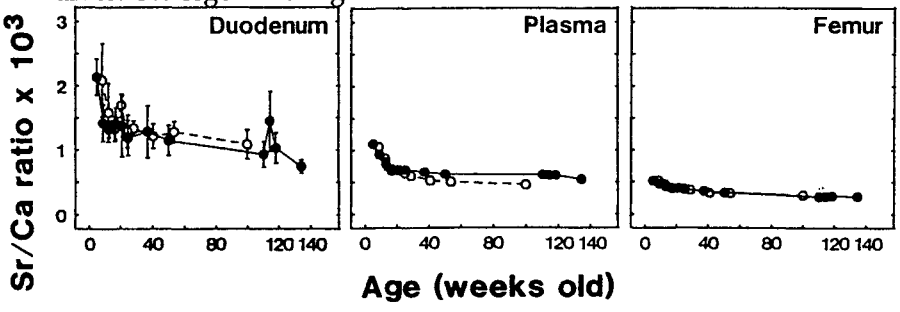

Fig. 4. Age-associated changes in Sr/Ca ratios in rat duodenum, plasma, and femur. Each data point represents mean ± SD for 3–5 rats. ●, male; ○, female.

values were smaller than zero (Table 1). Therefore, the Sr/Ca ratios were found to decrease with age with the same pattern in plasma, urine, and all tissues. In the femur, the correlation coefficient was the highest, and the standard error of estimate was the smallest among the tissues in each sex. In the duodenum, femur, and liver, no significant differences in the regression lines between the male and female rats were observed (Table 1).

DISCUSSION

In the present study, the age-associated changes in Sr/Ca ratio showed similar patterns in plasma, urine, and all tissues (Fig. 4–6) although a uniform relationship between age and the concentration of Ca or Sr was not observed among the tissues (Figs. 1–3).

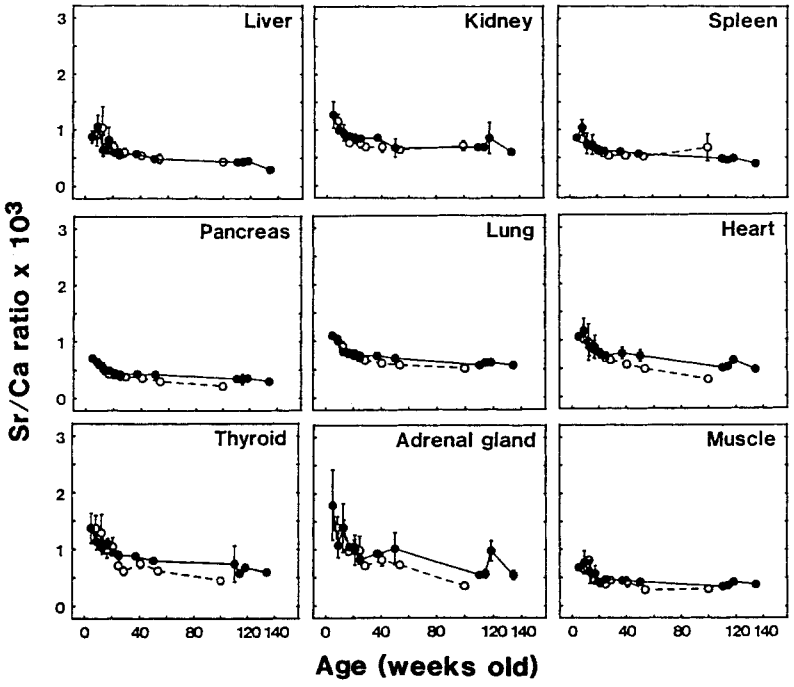

Fig. 5. Age-associated changes in Sr/Ca ratios in rat soft tissues. *See* legend to Fig. 4.

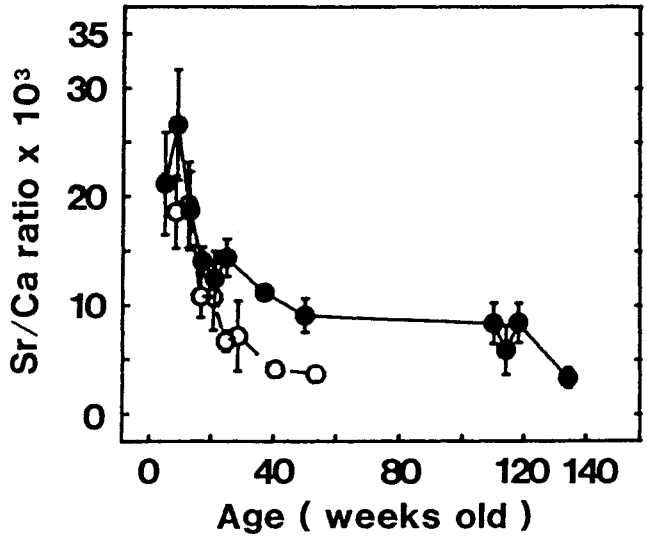

Fig. 6. Age-associated changes in Sr/Ca ratio in rat urine. *See* legend to Fig. 4.

Table 1
Parameters for Regression Equations[a]

	Male					Female					
	N^b	r^c	α	β	$S_y{}^d$	N^b	r^c	α	β	$S_y{}^d$	p^e
Duodenum	53	0.624	0.368	−0.183	0.111	43	0.616	0.388	−0.186	0.085	ns
Plasma	55	0.887	0.098	−0.167	0.042	43	0.946	0.205	−0.301	0.037	<0.001
Femur	55	0.988	−0.149	−0.195	0.015	43	0.970	−0.149	−0.200	0.018	ns
Liver	54	0.847	0.183	−0.282	0.085	43	0.770	0.171	−0.282	0.083	ns
Kidney	49	0.794	0.155	−0.155	0.058	43	0.771	0.149	−0.193	0.057	<0.001
Spleen	55	0.897	0.128	−0.227	0.054	43	0.596	0	−0.155	0.074	<0.01
Pancreas	54	0.879	−0.021	−0.224	0.058	42	0.913	0.042	−0.335	0.053	<0.001
Lung	52	0.945	0.144	−0.181	0.031	40	0.948	0.183	−0.250	0.030	<0.001
Heart	55	0.861	0.212	−0.231	0.065	43	0.894	0.323	−0.379	0.068	<0.001
Thyroid	55	0.885	0.289	−0.233	0.059	40	0.864	0.423	−0.385	0.081	<0.001
Adrenal gland	52	0.754	0.365	−0.267	0.111	43	0.839	0.465	−0.393	0.091	<0.001
Muscle	55	0.759	−0.024	−0.198	0.081	43	0.806	−0.079	−0.333	0.087	<0.001
Urine	54	0.851	1.769	−0.476	0.138	40	0.905	1.923	−0.797	0.117	<0.001

[a] $y = \alpha + \beta x$, where $y = \log (Sr/Ca \times 10^3)$ and $x = \log$ (age, wk).

[b] Number of observations. Because the data of contaminated specimens were eliminated, the numbers of observations in various tissues are not identical with one another.

[c] The correlation coefficient.

[d] The standard error of estimate.

[e] The evaluation of statistical differences between the regression lines of male and female rats; ns = not significant.

One of the most important physiological processes during which discrimination against Sr in favor of Ca has been known to occur is the intestinal absorption (2). Papworth and Patrick (*6*) suggested that Ca and Sr share a common carrier(s) that has a greater affinity for Ca than Sr and mediates the rate-limiting entry across the brush border. Comar described in his review (*4*) that the absorptive discrimination against Sr is smaller early in life and develops with age. The Sr/Ca ratios in the duodenum at 5 wk of age were near to the ratio in the diet (=2.43) in both male and female rats and thereafter decreased (Fig. 4). In addition, the levels of plasma Sr and Sr/Ca ratio were higher in the young rats (Figs. 1 and 4). These observations reflect high efficiency of Sr absorption by the small intestine early in life owing to a deficiency in absorptive discrimination. There was no evidence that absorptive discrimination altered in advanced age since no marked changes in the Sr/Ca ratio in the duodenum and plasma were observed in the rats over 2-yr-old (Fig. 4).

Numerous in vitro studies have shown that skeletal discrimination is small compared to other processes and overall operates to favor Ca retention in the skeleton (*7–9*). In the present study, the Sr/Ca ratios in the femur were lower than in plasma and other tissues in both male and female rats at all ages studied (Fig. 4), indicating that discrimination occurs, to some extent, under physiological conditions. Among the tissues studied, the relationship between the logarithm of the Sr/Ca ratio and the logarithm of age was most linear for femur (Table 1). Whether skeletal discrimination may alter with age cannot be evaluated from our results, because it is masked by the changes in large renal and intestinal discrimination.

The Sr/Ca ratios in the soft tissues could be owing to consequences of discrimination in various physiological processes. Since the patterns of age-related changes in the Sr/Ca ratio in all of the soft tissues were similar to those in the plasma (Fig. 5), they might be affected primarily by the changes in plasma. However, the parameters for regression lines (shown in Table 1) were different for tissues, suggesting the existence of specific discrimination mechanisms during the uptake processes of Ca and Sr by each tissue.

The mammalian kidney excretes Sr more rapidly than Ca (*10–12*). These elements are reabsorbed from the glomerular ultrafiltrate by the renal tubules, and the difference in rates of reabsorption between Ca and Sr is thought to be the major cause of renal discrimination (2). In our previous study, the ratio of the rate constant for Sr reabsorption to Ca reabsorption ($K = k_S / k_{Ca}$) has shown to be about 0.4 in the rat at 7 wk of age (*13*). For the clarification of the factors that brought about the age-associated changes in Sr/Ca ratio in the urine, as shown in Fig. 6, it is necessary to observe the K values for various ages.

The changes in Sr/Ca ratios with age observed in the present study might be produced by changes in discrimination during one or more physiological processes. Further studies will be needed to clarify the

effects of aging on the discrimination between Ca and Sr in each physiological process.

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