

Calcium-Strontium Discrimination by Rat Salivary Glands.* (24755)

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Since salivary glands appear distinct from soft tissues such as kidney and liver, in that they contain 4-5 times as much calcium(1), uptake and secretion by these glands of the chemically similar cation, strontium, has been studied to explore possible differences.

Methods. For comparative studies, 200-250 g female Long-Evans rats were given a measured dose of mixture of carrier-free Sr^{85} and high specific activity Ca^{45} as chlorides buffered for injection to pH 7.0 with 0.1 M sodium acetate. Injections were made into foot vein using ether anesthesia. An aliquot of injection mixture was analyzed to calculate percent of administered dose which appeared in tissue and blood samples. At intervals from 6 minutes to 16 hours, rats were etherized and samples of blood, kidney, parotid gland and submandibular gland with attached sublingual gland were removed for analysis in duplicate. Since preliminary experiments gave similar results for different salivary glands, in later experiments the 3 glands were pooled. Sr^{85} , which emits γ radiation of 0.013 and 0.513 MEV, was assayed on whole sample in well-type scintillation counter. At discrimination setting on this counter, it responded slightly to bremsstrahlung resulting from 0.254 MEV β of Ca^{45} , giving less than 1 count for each 500 counts indicated by GM counter used for Ca^{45} assay. Ca^{45} was assayed on same sample by the method previously described(1). The GM counter for Ca^{45} assay responded to γ radiation emitted by Sr^{85} , giving 1.4 counts for 100 counts registered by the scintillation counter. Thus, no correction was necessary for presence of Ca^{45} when assaying Sr^{85} . The Ca^{45} count was corrected if presence of Sr^{85} contributed more than 1% to total count. The data was reduced to percent of adminis-

tered dose appearing in sample. In additional rats, pilocarpine hydrochloride was given either 2 or 6 hours after administration of Ca^{45} - Sr^{85} mixture and 3 samples of saliva were collected from mouth over 2 hour interval. The rat was killed at end of 2 hour collection period and blood, kidney and salivary glands were removed for analysis of Ca^{45} and Sr^{85} . For comparison of Sr and Ca secretion, 2.5 meq/kg of .1 M strontium chloride were given intravenously over 5 minutes. After 2 hours, pilocarpine hydrochloride was administered subcutaneously and saliva was collected from submandibular duct. Three samples were obtained. At end of 2 hour collection, the rats were killed and blood, kidney and submandibular gland were removed for flame analysis of Ca as described previously (2). The 422.7 $m\mu$ line was used. Analysis of Sr on samples was also done by flame photometry using the 460.7 $m\mu$ line. Mutual interference between Ca and Sr was absent in this method.

Results. Table I gives percent of administered dose found in 100 mg of blood, kidney or salivary gland at various times after administration of Sr^{85} and Ca^{45} . No significant differences were found between blood and kidney and both showed a slightly more rapid disappearance rate for Sr^{85} than for Ca^{45} . Salivary glands showed retention of both Ca and Sr as compared to blood or kidney. Retention of Ca exceeded that of Sr at every time interval and reached a ratio of 4.66 to 1 at 16 hours.

Relative rates of secretion of Ca^{45} and Sr^{85} into saliva as compared to amounts in serum, kidney, and salivary glands are shown in Table II. Amounts of Ca^{45} and Sr^{85} in tissues are not significantly different from those in tissues when no pilocarpine was given (Table I). Amounts of Ca^{45} and Sr^{85} in saliva are slightly increased over serum at 4 hours, while at 8 hours, amount of Ca^{45} present is distinctly increased over serum, whereas amount of Sr^{85}

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TABLE I. Percent* of Administered Dose/100 mg Wet. Wt in Blood, Kidney and Salivary Gland at Various Times after I.V. Administration of $\text{Sr}^{85}\text{-Ca}^{45}$.

Time	No. rats	Blood			Kidney			Salivary glands		
		Ca^{45} , %	Sr^{85} , %	Ratio† Ca/Sr	Ca^{45} , %	Sr^{85} , %	Ratio† Ca/Sr	Ca^{45} , %	Sr^{85} , %	Ratio† Ca/Sr
6 min.	6	.135 $\pm .008$	.138 $\pm .007$	.99 $\pm .06$	.140 $\pm .014$	.123 $\pm .011$	1.13 $\pm .04$	.093 $\pm .011$	.073 $\pm .011$	1.32 $\pm .08$
1 hr	6	.042 $\pm .005$	.039 $\pm .005$	1.07 $\pm .07$	.047 $\pm .007$	.049 $\pm .006$	.95 $\pm .03$	.101 $\pm .014$	.044 $\pm .006$	2.27 $\pm .04$
4	6	.0133 $\pm .0011$	.0119 $\pm .0010$	1.15 $\pm .11$	.0164 $\pm .0011$	.0139 $\pm .0010$	1.20 $\pm .11$	.099 $\pm .002$	.036 $\pm .002$	2.77 $\pm .13$
8	5	.0065 $\pm .0006$	.0053 $\pm .0004$	1.33 $\pm .21$	.0081 $\pm .0005$	.0073 $\pm .0006$	1.14 $\pm .09$	.064 $\pm .007$	.023 $\pm .005$	3.00 $\pm .35$
16	5	.00036 $\pm .00004$	.00022 $\pm .00003$	1.62 $\pm .14$	.00032 $\pm .00006$	.00022 $\pm .00003$	1.44 $\pm .16$	.0019 $\pm .0003$	.00043 $\pm .00007$	4.66 $\pm .52$

* Mean $\pm$ stand. error.

† Mean of calculations for individual samples.

is the same as serum. Since this experiment was done with tracer amounts of Sr, a similar experiment was done with normal Sr to determine whether a large amount of Sr would make a difference in comparative secretion of Ca and Sr into the saliva. Concentrations of Ca and Sr in serum, kidney, submandibular gland and saliva after administration of strontium chloride are presented in Table III. Although concentrations of Ca and Sr in the submandibular gland differ markedly, the ratio of concentrations of Ca to Sr in saliva is approximately the same as in kidney and only slightly greater than that in serum.

Discussion. Recent work has shown that the body discriminates against strontium in favor of calcium in a number of ways. For example, Ca^{45} is preferentially absorbed from the gut by a factor of 1.6 over Sr^{89} (3). If Ca^{45} and Sr^{89} are injected together and concentrations of the 2 radio-isotopes in various parts of gastro-intestinal tract are followed, about twice as much Ca^{45} as Sr^{89} appears in bile and about 20% more Ca^{45} than Sr^{89} appears in remainder of gastro-intestinal tract(4). Under normal conditions, the fraction of injected Sr^{89} appearing in urine is greater than the fraction of injected Ca^{45} by a

TABLE II. Percent* of Administered Dose per 100 mg Wet Weight in Blood, Kidney, Salivary Gland and Saliva 4 and 8 Hours after $\text{Sr}^{85}\text{-Ca}^{45}$ I.V.†

Time, hr	Serum		Kidney		Salivary gland		Saliva	
	Ca^{45}	Sr^{85}	Ca^{45}	Sr^{85}	Ca^{45}	Sr^{85}	Ca^{45}	Sr^{85}
4	.0111 $\pm .0010$ (.88)	.0126 $\pm .0013$	.0143 $\pm .0006$ (.95)	.0150 $\pm .0011$	.098 $\pm .004$ (2.58)	.038 $\pm .003$	.0190 $\pm .0019$ (1.12)	.0170 $\pm .0022$
8	.0059 $\pm .0003$ (1.09)	.0054 $\pm .0005$	.0082 $\pm .0005$ (1.00)	.0082 $\pm .0007$	.071 $\pm .011$ (2.45)	.029 $\pm .006$	.0098 $\pm .0002$ (1.81)	.0054 $\pm .0003$

* Mean and stand. error for duplicate or triplicate samples from 3 rats at each time interval.

† Pilocarpine HCl (10 mg/kg) given S.C. 2 hr prior to autopsy. Ca/Sr ratio in parentheses.

TABLE III. Concentration* of Ca and Sr in Serum, Kidney, Submandibular Gland and Saliva 4 Hours after 2.5 meq/kg SrCl_2 I.V.†

Serum		Kidney		Submandibular gland		Saliva	
Ca	Sr	Ca	Sr	Ca	Sr	Ca	Sr
5.51 $\pm .20$ (1.25)	4.42 $\pm .23$	4.01 $\pm .26$ (1.51)	2.66 $\pm .36$	13.4 $\pm .4$ (5.4)	2.47 $\pm .22$	2.07 $\pm .11$ (1.68)	1.23 $\pm .09$

* Mean $\pm$ stand. error for duplicate or triplicate samples from 4 rats.

† Pilocarpine HCl (10 mg/kg) given S.C. 2 hr prior to autopsy. Ca/Sr ratio in parentheses.

factor of 1.6(5). The mechanism of this discrimination against Sr in favor of Ca has not been satisfactorily explained although Samachson and Lederer have shown that approximately 60% of Sr^{85} in human serum is filterable through a cellophane membrane as compared to 40% for Ca^{45} (6). Apparently bone is the only organ in which studies have been made of differential uptake of Ca and Sr and no difference in uptake appears to occur. However, other discriminatory processes in the body serve to lower the body burden and hence the bone burden of Sr more rapidly than Ca(7).

In our experiments, uptake of Sr^{85} by the salivary gland was distinctly less than that of Ca^{45} as shown at 1 hour after injection of a mixture of Ca^{45} - Sr^{85} (Table I). At this time, the percentage of administered dose of Ca^{45} in the gland was 2.4 times blood, whereas the percentage of injected Sr^{85} in the gland was only 1.13 times that in an equal quantity of blood. At the end of 8 hours after injection of the mixture of Sr^{85} - Ca^{45} , the percentage of administered dose of Sr remaining in the gland was 4.3 times the percent remaining in blood, while in contrast, the percent of administered dose of Ca remaining in the gland was 9.8 times that remaining in blood (Table I). Thus the process which retains Ca^{45} in the salivary glands(1) is less effective in retaining Sr^{85} .

The saliva secretory process discriminates against Sr less effectively than the process which retains Ca and Sr in the gland. Four hours after injection of the mixture, the percent of administered dose of Ca^{45} in saliva was 1.71 times serum whereas the gland/serum ratio for Ca^{45} was 8.8. In contrast, the percent of administered dose of Sr^{85} in saliva was 1.35 times serum and the gland/serum ratio for Sr^{85} was 3.0 (Table II). At 8 hours, saliva/serum ratio for Ca^{45} was 1.7 and the gland/serum ratio was 12.0. In contrast, saliva/serum ratio for Sr^{85} was 1.0 and gland/serum

ratio was 5.4. Thus, saliva/serum ratio in favor of Ca^{45} over Sr^{85} was 1.26 (1.7/1.35) at 4 hours and 1.7 (1.7/1.0) at 8 hours whereas gland/serum ratio in favor of Ca^{45} over Sr^{85} was 2.93 (8.8/3.0) at 4 hours and 2.23 (12.0/5.4) at 8 hours.

The data gained by injection of normal strontium demonstrates more strikingly the relative lack of discrimination against Sr in the secretion of saliva as compared to uptake of Sr by the submandibular gland (Table III). The Ca/Sr ratio in saliva (1.68) was only slightly different from serum (1.25), whereas the Ca/Sr ratio in the gland (5.4) was much larger.

Summary. 1) Percent of administered dose in blood, kidney, and salivary gland was determined from 6 minutes to 16 hours after intravenous injection of a mixture of Ca^{45} - Sr^{85} . Salivary glands retained Ca^{45} in preference to Sr^{85} , reaching a ratio of 4.6:1 at 16 hours. However, the glands showed a distinct retention of Sr^{85} and contained 3.1 times the concentration in kidney 8 hours after injection. 2) In secretion of saliva, the salivary glands showed less discrimination against Sr in favor of Ca than did uptake by the gland. After administration of tracer amount of Sr^{85} or of 2.5 meq/kg of normal Sr, ratio of Ca/Sr was only slightly greater in saliva than that in serum.

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