Calcium and Strontium Uptake by Rat Liver and Kidney Mitochondria.* (27814)

FRANK R. MRAZ†
Biology Laboratories, Hanford Laboratories, General Electric Co., Richland, Wash.

Vasington and Murphy(1) reported that calcium ions are accumulated against a concentration gradient by kidney mitochondria undergoing oxidative phosphorylation. DeLuca and Engstrom(2), however, showed that this process was not directly dependent upon oxidative phosphorylation, or upon the operation of the entire electron transport system, but that ATP, magnesium ions, and an oxidizable substrate were required. Dumont (3), using an *in vivo* preparation of rat small intestine, found that in the concentration range of 0 to 25 mM, calcium transport could not be ascribed to passive independent movement of calcium ions, while strontium flux appeared to be passive. Mraz(4) reported that calcium reduced absorption of Ca^{45} by the small intestine to a greater degree than it reduced the absorption of Sr^{85}, while strontium reduced Sr^{85} and Ca^{45} absorption slightly, but did not appreciably affect the ratio of Sr^{85} to Ca^{45} absorbed. It was of interest to determine whether the effect of calcium and strontium on uptake of Sr^{85} and Ca^{45} by liver and kidney mitochondria follows a similar pattern to that found in intestinal absorption.

Methods. Sprague-Dawley female rats with an average weight of 200 g were fed a commercially pelleted diet containing 1.3% Ca, 0.9% P and 5 U.S.P. units of vit D per gram of diet, for at least 20 days prior to sacrifice. They were killed by a sharp blow on the head followed by decapitation. The kidneys and liver were removed quickly and chilled in ice-cold 0.25 M sucrose solution. A 10% homogenate of the tissues was prepared at 0°C with a Potter-Elvehjem homogenizer. The homogenate was centrifuged at 800 × *g* for 10 minutes to remove nuclei, debris, and broken cells. The upper layer was removed carefully and centrifuged at 12,000 × *g* for 10 minutes to sediment the mitochondria. The preparation was washed once with 0.25 M sucrose and finally suspended in 5 ml of 0.25 M sucrose. One-half milliliter of this suspension was incubated for 10 minutes at 30°C in the mixture used by DeLuca and Engstrom(2) minus the added calcium and plus 0.16 μc each of Ca^{45} (0.0036 mg Ca as the chloride) and Sr^{85} (0.0162 mg Sr as the nitrate). Unless otherwise indicated the incubation was conducted in a Dubnoff-type shaker with air as the gas phase. After incubation the mixture was rapidly chilled to 0°C and immediately centrifuged at 42,000 × *g* for 7 to 10 minutes. The supernatant was decanted and analyzed for Ca^{45} and Sr^{85}.

The effect of varying calcium and/or strontium concentration in the medium, on uptake of Ca^{45} and Sr^{85} by mitochondria, was tested in the first experiment. In the second experiment, the necessity of ATP and cytochrome c, for uptake of Ca^{45} and Sr^{85} by mitochondria from rat liver and kidney, was determined in presence and absence of calcium. In all experiments at least 4 samples from each of a minimum of 3 animals were used. All samples were run with controls containing either no calcium or 9 μM calcium depending upon the comparisons made.

Results and discussion. In the first experiment (Table I), absorption of Sr^{85} was less than that of Ca^{45} in every instance. Addition of 0.9 μM calcium to the medium decreased both Ca^{45} and Sr^{85} uptake. Sr^{85} was reduced to a greater degree than Ca^{45}, thus decreasing the ratio of Sr^{85} to Ca^{45} absorbed. The reverse was true when 0.9 μM of strontium was added to the medium, with no calcium added; both Sr^{85} and Ca^{45} were increased, with the increase in Sr^{85} being greater. Addition of equal amounts of strontium and calcium to the medium increased Sr^{85} and Ca^{45} uptake to the extent observed with

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† Exchange scientist from University of Tennessee-Atomic Energy Commission Agric. Research Lab., Oak Ridge.

TABLE I. Effect of Strontium and Calcium on Absorption of Sr^{85} and Ca^{45} by Kidney and Liver Mitochondria.

Variables		Kidney*			Liver*		
Ca	Sr	Sr^{85}	Ca^{45}	Ratio Sr^{85}/Ca^{45}	Sr^{85}	Ca^{45}	Ratio Sr^{85}/Ca^{45}
(μM)		(% dose)			(% dose)		
.0	.0	36 ±10	54 ±13	.67	63 ±12	80 ± 7	.79
.9	.0	19 ± 2	40 ± 4	.48	13 ± 3	40 ± 4	.32
.0	.9	82 ± 2	91 ± 1	.90	82 ± 4	92 ± 3	.89
.225	.225				74 ± 9	88 ± 2	.84
.45	.45				78 ± 6	90 ± 1	.87
.9	.9				77 ± 7	92 ± 0	.84

* Mean values ± stand. dev.

TABLE II. Effect of Calcium, Cytochrome c and ATP on Kidney and Liver Mitochondrial Absorption of Ca^{45} and Sr^{85}.

Variables			Kidney*			Liver*		
Ca	ATP	Cyto-chrome c	Sr^{85}	Ca^{45}	Ratio Sr^{85}/Ca^{45}	Sr^{85}	Ca^{45}	Ratio Sr^{85}/Ca^{45}
(μM)			(% dose)			% dose		
.0	6	.08	36 ±10	54 ± 13	.67	63 ± 12	80 ± 7	.79
.0	6	.00	40 ± 7	68 ± 11	.59	55 ± 30	67 ± 37	.82
.0	0	.08	4 ± 2	0 ± 0	—	58 ± 32	71 ± 25	.82
.9	6	.08	19 ± 2	40 ± 4	.48	13 ± 3	40 ± 4	.32
.9	6	.00	21 ± 6	49 ± 12	.43	8 ± 4	14 ± 14	.57
.9	0	.08	2 ± 2	0 ± 0	—	3 ± 1.8	2 ± 4	1.50

* Mean values ± stand. dev.

strontium alone. It would appear that the presence of strontium prevented the expression of the calcium effect.

In the second experiment (Table II) ATP was shown to be necessary for uptake of Ca^{45} and Sr^{85} by kidney mitochondria, in both presence and absence of stable calcium, while cytochrome c was found unnecessary. This is in agreement with the findings of DeLuca and Engstrom(2) on calcium. In the absence of added calcium, the liver mitochondria would appear to require neither ATP nor cytochrome c for uptake of Ca^{45} or Sr^{85}. The large standard deviations obtained in these experiments suggest that sufficient residual ATP and cytochrome c may be present in the liver preparation to account for the movement of the small amounts of calcium and strontium present in the medium. With addition of 0.9 μM of calcium, ATP appeared to be required for both Ca^{45} and Sr^{85} uptake, while cytochrome c appeared to be required only for Ca^{45} uptake.

The changes observed in the ratio of Sr^{85} to Ca^{45} uptake, when calcium or strontium was added, indicate that their uptake by the mitochondria occurs by different routes. The fact that this ratio decreases with addition of calcium, and increases with addition of strontium, even in the presence of added calcium, suggests that the mechanisms responsible for the preferential absorption of calcium are interfered with by strontium. It may well be that the mitochondria are responsible for the increase in total strontium absorption that is found when stable strontium is included in the diet of animals(3,4,5, 6).

Summary. The uptake of Sr^{85} by mitochondria from rat kidney and liver was reduced to a greater degree by added calcium than was the uptake of Ca^{45}. Uptake of Sr^{85} was increased to a greater degree by addition of strontium than was the uptake of Ca^{45}. ATP was required for the uptake of Sr^{85} and Ca^{45} by kidney mitochondria, while cytochrome c was not. Results with liver mitochondria were more variable and the requirement for ATP appeared to be influenced by calcium in the medium.

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Gastrointestinal Sites of Absorption and Endogenous Secretion of Calcium and Phosphorus in Dairy Calves.* (27815)

P. T. CHANDLER AND R. G. CRAGLE (Introduced by F. R. Mraz)
Agricultural Research Laboratory, University of Tennessee, Oak Ridge,† and Dairy Department, University of Tennessee, Knoxville

Much work has been conducted concerning the total absorption of calcium and phosphorus. The sites within the gastrointestinal tract where absorption and secretion occur are not well known. The ideal method for studying gastrointestinal absorption and secretion is the use of non-absorbed indicators that move through the tract as does the nutrient in question. Absorption and secretion of calcium and phosphorus have been studied using reference markers of iron oxide(1), titanium dioxide(2,3,4), and insoluble ash of feed(5). Garner *et al.*(6) studied cerium-144 metabolism in the dairy cow and suggested its use as a marker in digestibility trials. In the present study, gastrointestinal absorption and secretion of calcium and phosphorus in the diary calf have been investigated using Ce^{144} as a non-absorbed marker.

Method. Eight Holstein male calves, 12 and 15 weeks of age, were used. The animals were raised on skim milk and calf starter ration. Reconstituted skim milk (13% solids) was fed at the rate of 10 lb per 100 lb of body weight per day until 4 weeks of age. Over the next 2 weeks the skim milk was gradually reduced to zero. The starter consisted of 15% alfalfa meal, 10% dried skim milk, 25% soybean oil meal, 10% oats, 38% yellow corn, 1% salt, and 1% vit. A and D supplement. The calves were fed this starter throughout the experiment, and at time of termination they were eating 3 lb per day.

The animals were divided into 2 groups of 4 each and trained to stand at ease in metabolism stalls. At 12 weeks of age, Group 1 was placed in the stalls and fed 1.5 lb of starter plus 50 μc of Ce^{144} twice daily. This feeding rate supplied an average of 8.05 g calcium, 6.44 g phosphorus and 10.08×10^6 cpm Ce^{144} per day per calf. After 7 days on this regime the tract contents approached equilibrium (Fig. 1). The animals were then slaughtered and digestive contents sampled. The contents sampled consisted of the rumen, omasum, abomasum, small intestines, cecum, coiled colon, and posterior colon. At 15 weeks of age, Group 2 was placed in stalls and dosed similarly to Group 1.

The Ce^{144} was blended into the starter by making a pre-mix of starter plus isotope and then mixing the pre-mix and feed together before feeding. The pre-mix was mixed by adsorbing the isotope on 50 g of anion exchange resin (Amberlite IRA-400, 20-50 mesh) and then mixing with 950 g of feed. An anion exchange resin was used to provide an adsorbing surface and to offer no competition for the cation. All mixing was carried out by tumbling the material in glass jars.

At slaughter, the gastrointestinal tract was tied off and removed from the animal as soon as possible. The tract was then dissected

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