

The Effects of Sodium Alginate and Other Untested Polymers on Radiostrontium Retention in the Rat¹ (34666)

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Concern over radiostrontium is understandable because of its substantial fission yield, long half-life, facile incorporation into biological systems, and striking chemical and biological similarities to calcium. Once absorbed, radiostrontium, similar to calcium, becomes incorporated primarily into the skeletal system where its penetrating beta radiation into skeletal tissue and hematogenic bone marrow may produce osteosarcomas (1) and leukemias (2).

To date, no radioprotective agents are available which are nutritionally adequate and effective against the absorption and retention of radiostrontium. Chelating agents and ion exchange resins are potentially useful against strontium absorption and retention, but so far have been largely ineffective. This disappointing situation has been reviewed and discussed by several authors (3, 4). The crux of the matter has been that the chelating agents and ion exchange resins evaluated bind calcium more favorably than strontium and thus alter calcium metabolism much more drastically than strontium metabolism.

However, sodium alginate, a nonabsorbable, polyelectrolytic seaweed extract, has been shown to substantially and *selectively* reduce radiostrontium intestinal absorption and skeletal retention in rats (5) and man (6) without significantly altering calcium metabolism. Alginate is a polymer of D-mannuronic and L-guluronic acids. As the L-guluronic acid content of the alginate polymer increases, the ability to preferentially bind strontium also increases (7). This study was performed to obtain additional data concern-

ing the effects of alginate and other untested polymers on radiostrontium absorption and retention in the rat.

Experimental Method. A total of 140 male rats (100 – 110 g, Wistar)² was divided into 10 equal groups according to body weight. Each animal was weighed individually and earmarked. All animals were housed in a temperature-controlled room in raised wire-bottom cages containing 2 animals/cage and provided distilled fluoride-free drinking water and a stock corn diet³ with or without supplementary agents *ad libitum*.

Radiostrontium (⁸⁹Sr) and radiocalcium (⁴⁵Ca) (5 μCi of ⁸⁹Sr; 3 μCi of ⁴⁵Ca) as the chlorides were administered by orogastric intubation to each rat after the animals had consumed the experimental diets for 72 hr. After 48 additional hours of ingesting the experimental diets, the animals were sacrificed by chloroform inhalation. The femurs and carcasses were individually ashed and analyzed for ⁸⁹Sr and ⁴⁵Ca (8).

All radioactive samples were counted twice, once with a 71.9 mg/m² aluminum absorber placed between the detector and the radiosample, and once without the absorber in order to distinguish between the weaker beta emission of ⁴⁵Ca and the stronger beta emission of ⁸⁹Sr. No corrections for radioactive decay were necessary as experimental samples, standards and controls were always assayed within a 48-hr period. The major error sources inherent in these radioactive

² Harlan Industries, Cumberland, Indiana.

³ This diet is composed as follows (%): yellow corn meal, 64.0; powdered whole milk, 30.0; alfalfa meal, 4.8; iodized salt, 1.0; and irradiated yeast, 0.2. Supplemental protective agents were added at the expense of the yellow corn meal.

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measurements are the dilutions and aliquots taken for experimental samples and standard solutions. Radioactivity measurements were determined with an end-window Geiger-Mueller radiation detector tube in an automatic sample changer.⁴ The samples were counted for a sufficient number of counts in order to reduce the probable error due to statistical variation of counting to less than 5%. The detection system efficiency was 5.17% for ⁸⁹Sr and 6.89% for ⁴⁵Ca.

Statistical evaluation of the data was obtained using the analysis variance with the repeated *t* test procedure used to test for significant differences between individual group means. The Bartlett chi-square test for homogeneity was used and, in cases in which homogeneity could not be assumed, the Welch test modified this variable.

The radioactive data are expressed as percentages of intubated dose (pid). All results are presented minus background radiation and minus nonradioactive control animal values. The observed ratio (9) or discrimination originally developed to quantitate differences between the metabolism of radiostrontium and stable calcium has been altered to compare both radioisotopes side by side from the precursor and experimental samples, so as to compensate for any sudden changes in stable calcium ingestion and its subsequent metabolism.

The groups examined were as follows: Group A, nonradioactive control fed the stock corn diet; and Group B, ⁸⁹Sr ⁴⁵Ca-control plus the stock corn diet. The remaining animals received the ⁸⁹Sr ⁴⁵Ca plus the stock corn diets to which the following levels of experimental additives were added: Group C, 1% sodium alginate⁵; Group D, 5% sodium alginate; Group E, 10% sodium alginate; Group F, 1% guar⁶; Group G, 5% guar; Group H, 10% guar; Group I, carboxymethylguar⁷; Group J, 5% carboxymethylguar; Group K, 10% carboxymethylguar; Group

L, 1% Questran⁸; Group M, 5% Questran; and Group N, 10% Questran.

Results. Table I shows the femur radiocontamination retention results. The ash weight data show that the nonradioactive control animals had a mean ash weight of 0.155 g and that none of the radioactive experimental animal groups have values significantly different. The radiocontrol animals had a mean femur ⁸⁹Sr concentration value of 10.27 pid/g ash. The animals receiving alginate (Group C through E) showed a marked reduction in femur concentration of radiostrontium with values of 5.28, 4.73, and 3.14 pid/g ash, respectively. The remaining experimental groups indicated no change to increased ⁸⁹Sr femur concentration with values that ranged from 9.40 pid/g ash (Group G) to 15.15 pid/g ash (Group I).

The radiocontrol animals retained a mean value of 1.63 pid ⁸⁹Sr in femurs. The total ⁸⁹Sr femur retention values for the animals which received the dietary additives ranged from 0.48 pid (Group E) to 2.33 (Group I).

The radiocalcium femur values were uniformly higher than those for the corresponding radiostrontium values. The radiocontrol animals had a mean ⁴⁵Ca femur concentration value of 12.9 pid/g ash weight. The mean values for the experimental groups ranged from 12.8 to 24.3 pid/g ash weight (Group C and J, respectively). Total radiocalcium femur retention values ranged from 1.80 pid (Group C), 2.06 pid (Group B, radiocontrol animals), to 3.68 pid (Group J).

The femur discrimination factor is the ratio of ⁸⁹Sr/⁴⁵Ca in the femur to ⁸⁹Sr/⁴⁵Ca in the intubated dose. The femur discrimination values ranged from 0.10 (Group E) to 0.36 (the radiocontrol animals) with the remaining experimental groups between these extremes.

The carcass radioisotope retention results are summarized in Table II. Generally, the carcass data paralleled those of the femur. None of the experimental groups' mean carcass ash weight were significantly different from the nonradioactive control animals' average value of 3.11 g.

⁴ Nuclear-Chicago, Model C-110B.

⁵ Fisher Scientific Company (Kelco, Inc.).

⁶ Stein, Hall & Company, Inc. (courtesy of Mr. Max Goldfrank).

⁷ General Mills, Inc. (courtesy of Mr. D. E. Terry).

⁸ Mead Johnson Laboratories.

TABLE I. A Summary of Radiostrontium and Radiocalcium Femur Retention Results.

Group	Treatment	Ash wt	Radiostrontium			Radiocalcium			Sr pid/Ca pid /femur	Discrim. ^b
			pid/g	pid/femur	pid/g	pid/femur	pid/femur			
A	Control	0.155 ± 0.025 ^a	—	—	—	—	—	—	—	
B	Control + ⁸⁵ Sr, ⁴⁵ Ca	0.157 ± 0.028	10.27 ± 2.49	1.63 ± 0.54	12.9 ± 2.8	2.06 ± 0.67	0.81 ± 0.17	0.36		
C	1% Alginate + ⁸⁵ Sr, ⁴⁵ Ca	0.142 ± 0.027	5.28 ± 1.44	0.87 ± 0.29	12.8 ± 1.9	1.80 ± 0.31	0.42 ± 0.15	0.19		
D	5% Alginate + ⁸⁵ Sr, ⁴⁵ Ca	0.144 ± 0.029	4.73 ± 0.76	0.68 ± 0.17	16.2 ± 4.7	2.34 ± 0.58	0.29 ± 0.06	0.13		
E	10% Alginate + ⁸⁵ Sr, ⁴⁵ Ca	0.149 ± 0.030	3.14 ± 1.35	0.48 ± 0.24	13.8 ± 5.7	1.98 ± 0.94	0.23 ± 0.12	0.10		
F	1% Guar + ⁸⁵ Sr, ⁴⁵ Ca	0.152 ± 0.025	13.60 ± 2.97	2.07 ± 0.27	22.7 ± 5.9	3.42 ± 0.90	0.62 ± 0.11	0.27		
G	5% Guar + ⁸⁵ Sr, ⁴⁵ Ca	0.148 ± 0.027	9.40 ± 2.17	1.37 ± 0.33	15.6 ± 4.2	2.27 ± 0.54	0.62 ± 0.11	0.27		
H	10% Guar + ⁸⁵ Sr, ⁴⁵ Ca	0.138 ± 0.020	9.97 ± 2.24	1.35 ± 0.27	13.8 ± 2.6	1.86 ± 0.25	0.73 ± 0.09	0.32		
I	1% CM Guar + ⁸⁵ Sr, ⁴⁵ Ca	0.156 ± 0.025	15.15 ± 5.38	2.33 ± 0.89	21.1 ± 5.9	3.26 ± 1.10	0.72 ± 0.20	0.31		
J	5% CM Guar + ⁸⁵ Sr, ⁴⁵ Ca	0.152 ± 0.024	13.69 ± 2.13	2.07 ± 0.37	24.3 ± 3.7	3.68 ± 0.67	0.58 ± 0.13	0.25		
K	10% CM Guar + ⁸⁵ Sr, ⁴⁵ Ca	0.160 ± 0.029	13.44 ± 3.58	2.17 ± 0.72	21.0 ± 2.8	3.34 ± 0.56	0.65 ± 0.13	0.29		
L	1% Questran + ⁸⁵ Sr, ⁴⁵ Ca	0.152 ± 0.025	14.11 ± 2.88	2.13 ± 0.49	22.1 ± 5.4	3.27 ± 0.60	0.67 ± 0.19	0.29		
M	5% Questran + ⁸⁵ Sr, ⁴⁵ Ca	0.150 ± 0.032	10.30 ± 2.91	1.51 ± 0.39	14.4 ± 4.1	2.11 ± 0.53	0.73 ± 0.11	0.32		
N	10% Questran + ⁸⁵ Sr, ⁴⁵ Ca	0.138 ± 0.026	14.6 ± 4.23	2.00 ± 0.57	21.1 ± 5.4	2.87 ± 0.72	0.70 ± 0.14	0.31		

^a Mean values ± standard deviation.^b Discrimination = $\{[^{85}\text{Sr}/^{45}\text{Ca}(\text{femur})]/[^{85}\text{Sr}/^{45}\text{Ca}(\text{intubated dose})]\}$: Actual intubated dose ratio = 2.277.

TABLE II. A Summary of Radiostrontium and Radiocalcium Carcass Retention Results.

Group	Treatment	Ash wt	Radiostrontium		Radiocalcium		Sr pid/Ca pid /carcass	Discrim. ^b
			pid/g	pid/carcass	pid/g	pid/carcass		
A	Control	3.12 ± 0.33 ^a	—	—	—	—	—	—
B	Control + ⁸⁵ Sr, ⁴⁵ Ca	3.25 ± 0.24	9.79 ± 1.54	31.7 ± 4.3	12.6 ± 2.8	34.5 ± 8.5	0.68 ± 0.12	0.34
C	1% Alginat + ⁸⁵ Sr, ⁴⁵ Ca	3.14 ± 0.43	7.62 ± 2.37	23.8 ± 7.4	17.6 ± 4.2	46.6 ± 13.8	0.41 ± 0.08	0.20
D	5% Alginat + ⁸⁵ Sr, ⁴⁵ Ca	3.09 ± 0.30	10.27 ± 2.42	31.7 ± 8.4	16.9 ± 3.3	43.7 ± 14.1	0.58 ± 0.09	0.29
E	10% Alginat + ⁸⁵ Sr, ⁴⁵ Ca	3.08 ± 0.31	5.13 ± 2.01	15.9 ± 6.6	19.3 ± 7.0	59.6 ± 18.5	0.21 ± 0.11	0.10
F	1% Guar + ⁸⁵ Sr, ⁴⁵ Ca	3.05 ± 0.30	14.09 ± 1.72	42.6 ± 5.7	19.1 ± 4.6	57.8 ± 7.7	0.64 ± 0.11	0.32
G	5% Guar + ⁸⁵ Sr, ⁴⁵ Ca	3.02 ± 0.40	9.08 ± 1.15	27.1 ± 2.7	13.0 ± 1.7	38.9 ± 4.7	0.63 ± 0.13	0.31
H	10% Guar + ⁸⁵ Sr, ⁴⁵ Ca	2.91 ± 0.32	7.18 ± 3.22	20.8 ± 9.4	8.7 ± 1.4	25.2 ± 8.4	0.80 ± 0.16	0.39
I	1% CM Guar + ⁸⁵ Sr, ⁴⁵ Ca	3.12 ± 0.34	11.07 ± 3.29	33.9 ± 9.4	13.1 ± 2.8	40.0 ± 11.7	0.80 ± 0.12	0.39
J	5% CM Guar + ⁸⁵ Sr, ⁴⁵ Ca	3.05 ± 0.21	9.68 ± 2.75	29.3 ± 7.5	14.2 ± 3.2	46.1 ± 10.8	0.62 ± 0.10	0.31
K	10% CM Guar + ⁸⁵ Sr, ⁴⁵ Ca	3.10 ± 0.36	10.41 ± 1.78	32.1 ± 5.7	14.3 ± 2.1	44.5 ± 9.7	0.74 ± 0.15	0.37
L	1% Questran + ⁸⁵ Sr, ⁴⁵ Ca	3.08 ± 0.28	11.13 ± 2.19	33.9 ± 4.6	14.5 ± 3.9	44.1 ± 12.2	0.78 ± 0.17	0.39
M	5% Questran + ⁸⁵ Sr, ⁴⁵ Ca	3.05 ± 0.44	8.64 ± 1.74	25.9 ± 4.3	10.8 ± 2.6	32.4 ± 5.4	0.81 ± 0.20	0.40
N	10% Questran + ⁸⁵ Sr, ⁴⁵ Ca	2.92 ± 0.27	11.07 ± 2.28	32.1 ± 6.0	14.1 ± 3.1	41.0 ± 7.4	0.76 ± 0.14	0.38

^a Mean values ± standard deviation.^b Discrimination = $\left\{ \frac{[^{85}\text{Sr}/^{45}\text{Ca} (\text{carcass})]}{[^{85}\text{Sr}/^{45}\text{Ca} (\text{intubated dose})]} \right\}$: Actual intubated dose ratio = 2.081.

The radiocontrol animals' mean carcass ^{89}Sr concentration value was 9.79 pid/g ash. Only two experimental groups showed much variation. The Group E animals had 5.13 pid/g ash, while 14.09 pid/g ash was found for Group F. Understandably, these same two groups had markedly different total carcass ^{89}Sr mean retention values from the radiocontrol value of 31.7 pid, with values of 15.9 and 42.6 pid, respectively.

The average carcass ^{45}Ca concentration of the radiocontrol animals was 12.6 pid/g ash. The animals receiving alginate had numerically, but not significantly, increased levels of carcass ^{45}Ca with mean values of 17.6, 16.9, 19.3 pid/g ash (Groups C-E), respectively. The smallest radiocalcium carcass concentration value was 8.7 pid/g ash (Group H). The total mean radiocalcium carcass accumulation for the radiocontrol animals was 34.5 pid, the largest carcass accumulation value was 59.6 pid (Group E), and the lowest was 25.2 pid (Group H). The carcass discrimination values ranged from 0.40 (Group M) to 0.34 (the radiocontrol group) to 0.10 (Group E).

Discussion. Since the mean ash weight values for femur and carcass were not significantly different between individual groups, the critical results were the ^{89}Sr and ^{45}Ca concentration and discrimination values. The femur results indicated that alginate was the only additive effective in reducing skeletal ^{89}Sr deposition. The alginate (Groups C, D, E) had femur ^{89}Sr values 50, 55, and 70%, respectively, less than the radiocontrol animals (significant at 0.01). The corresponding ^{45}Ca values for the alginate animals were essentially the same as those for the radiocontrol animals.

This selective action toward strontium by alginate was obvious in the discrimination values. The normal biological discrimination represented by the radiocontrol animals' discrimination value was suggested to be nearly one-half as efficient as that of the 1% alginate (Group C), one-third that of the 5% alginate (Group D), and one-fourth that of the 10% alginate (Group E). None of the other dietary additives showed any significant improvement in strontium-calcium

discrimination as compared to the radiocontrol value; the numerically improved discrimination values were apparently due to slightly elevated ^{45}Ca retention.

The carcass results generally paralleled those of the femur. The animals receiving 10% alginate showed 50% less carcass ^{89}Sr (significant at 0.01), essentially the same carcass ^{45}Ca , and nearly 3-fold improved discrimination when compared to the radiocontrol animals. The animals in Groups C and D which received 1 and 5% alginate have carcass ^{89}Sr and ^{45}Ca values essentially no different from the radiocontrol values. The animals were housed in pairs, sacrificed by group, and were not skinned before ashing. Thus, the differences between femur and carcass results, in this instance, could be due to pelt radiocontamination. The remaining dietary agents, guar gum, carboxymethyl guar gum, and Questran showed no differences in carcass ^{89}Sr and ^{45}Ca values when compared to the radiocontrol values.

Since guar gum is a polymer of galactose and mannose, carboxymethyl guar gum is its carboxymethyl ether derivative, Questran is the chloride salt of cholestyramine, and alginate is a polymer of guluronic and manuronic acids, it seems probable that the free carboxylic functions in alginate contribute to the binding of radiostrontium. It is known that as the L-guluronic acid content of alginate is increased, the selectivity toward strontium is enhanced (7). It would be desirable to test polymers of pure L-guluronic acid and determine what the critical chain length would be for optimal strontium binding and yet be nonabsorbable from the intestinal tract.

Summary. At levels of 1, 5, and 10%, sodium alginate, guar gum, carboxymethyl guar gum, and Questran were tested in diets fed to rats to determine their effects on radiostrontium skeletal retention. Alginate was the only effective agent against radiostrontium retention, approaching, at the 10% level, 70% less ^{89}Sr skeletally retained than radiocontrol animals and a 3-fold improvement over natural biological strontium-calcium discrimination. The strontium-binding ability

shown by alginate was probably due to its functional carboxyl groups.

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