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Research Article

INFLUENCE OF PECTIN SUBSTANCES ON STRONTIUM REMOVAL IN RATS

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ABSTRACT

Exposure to strontium isotopes remains a widespread problem in most industrialized countries. Modern agents purposed for prevention of the radioisotope absorption as well as for therapy of chronic poisoning with these substances generally exert toxic effects causing mineral misbalance. Dietary non-starch polysaccharides were suggested as substances effectively binding bivalent ions. The present study was carried out to estimate strontium binding capacity of low and high esterified pectin compounds and their influence on strontium absorption, distribution, and removal in laboratory rats. Under in vitro conditions, pectin with low degree of esterification was the most effective and bound 2.2 mmol of strontium ions per gram. Administration of low esterified pectin and its calcium salt in rats resulted in significantly reduced amount of strontium accumulated in liver, heart, kidney, and femur. After advance administration of the strontium chloride solution the low esterified pectin and calcium pectate contributed to the removal of the strontium ions from organs and femur in rats. All their effects were more pronounced than those of high esterified pectin. These effects were also proved by 45.7% and 21.0% increased amount of strontium being eliminated with feces of the animals given respectively calcium pectate and low esterified pectin. The results obtained show that the low esterified pectin and calcium pectate may be considered as the perspective dietary components protecting humans from environmental strontium.

Keywords: Pectin, Chemical properties, Strontium, Elimination, Rats.

INTRODUCTION

Nuclear energy is becoming a preferred energy source amidst rising concerns over the impacts of fossil fuel based energy on global warming and climate change. The radioactive waste generated by the nuclear power plants contains harmful long-lived fission products including ions of strontium[1]. Long-term environmental contamination with strontium may raise some concern due to its potential transfer to humans through the plant-animal-human food chain causing general population exposure[2]. Thus humans can be exposed to strontium by eating food or drinking water containing this metal[3]. After entering a body with contaminated food or water, most of the strontium amount is generally excreted, but part of it remains fixed in bones and bone marrow as well as in blood and soft tissues. Also being in bloodstream strontium may be distributed throughout the whole body easily entering and leaving cells. Biochemical behavior of strontium is similar to other bivalent metals such as calcium. Strontium is a beta-emitter and a large source of Cherenkov radiation and its presence in bones can cause bone cancer, cancer of nearby tissues, and leukemia[4]. Therefore, the development of simple and effective materials preventing absorption of strontium in intestine and accelerating its removal from human body is a topic of current interest[5].

It was suggested previously that pectin-rich fruits as well as isolated pectin materials are effective agents removing metal ions as from water solutions[6] as from human body[7]. Pectin substances belonging to the group of natural biopolymers are the ionic plant polysaccharides, which main structural features are the linear chains containing more than 100 (l-4)-linked α-D-galacturonic acid residues[8]. Non-starch polysaccharides are often used in medicine because of their healing proprties[9]. Their metal binding capacity was proved in the numerous studies under as *in vivo* as *in vitro* conditions[7,10,11]. The main structural characteristic of all pectin substances is their degree of esterification meaning the number of galacturonic acid residues in their molecules with the methanol radical attached. Previously the "egg-box" model was proposed for description of the metal binding mechanism of pectins[12], stating that esterified residues of galacturonic acids are not active whereas negative charges of the free carboxyl groups in pectin molecules form covalent bonds with the metal ions. Therefore it may be presumed that pectin with the low degree of esterification exerts considerably higher metal binding activity[13]. Also interaction with metals is dependent on the rheological properties of pectin determining the rate of diffusion of ions into polysaccharide gel[14].

In this study the influence of pectins with high and low degree of esterification on the absorption, retention and elimination of Sr^{2+} was investigated. Before experiments strontium binding capacity of the pectin substances was assessed under in vitro conditions.

MATERIALS AND METHODS

Materials

Pure strontium chloride was purchased from Sigma Chemical (St. Louis, Mo, USA). All other chemicals were of the highest quality available. Citrus pectin marked as Classic CS501 without additives was obtained from Herbstreight & Fox, Germany. The pectin preparation contained no acetyl or amide groups. This pectin was used for preparation of the low-esterified pectin and calcium pectate. For the de-esterification process 100 g of high-esterified commercial pectin was treated with 1600 mL 50% ethanol containing 20 g NaOH and 20 g KOH and low-esterified pectin was isolated by filtration. Calcium pectate was prepared from 100 g of low-esterified pectin suspended in 500 mL 70% ethanol with addition of $CaCL_2 \cdot 6H_2O$.

The galacturonan content in the pectins was determined by m-hydroxydiphenyl method[15]. The degree of esterification was characterized using titrimetric analysis with Hintone indicator[16]. Intrinsic viscosity of pectins was determined in 0.05 mol/L NaCl per 0.005 mol/L Na-oxalate at 25.0°C and pH 6.0 using an Ubbelohde viscosimeter. Then intrinsic viscosity was related empirically to the molecular weight by the Mark-Howink relation[17]. The calcium content in the calcium pectate was assayed by atomic-absorption method[18].

Estimation of the strontium binding capacity

Metal binding capacity of the pectin samples was assessed using the test tubes containing 1000 mL of 0.2×10^{-3} mol/L and 2.0×10^{-3} mol/L strontium chloride solutions. 500 mg of each of the polysaccharide samples were added into the test tubes and then incubated for 24 hours. pH of the media was 5.0, temperature of solutions was 20°C, and the steering speed was 400 rpm. In 24 hours the strontium solution containing pectin compounds was filtered and strontium content in the supernatant fluid was determined using titrimetric method. Strontium binding capacity of the samples was calculated as the difference between the metal concentrations in the initial solution and the one in the supernatant.

Animals and diet

Male Wistar rats (Laboratory of Pharmacology, Institute of Marine Biology, Vladivostok, Russia) weighing 130 to 160 g were housed in stainless steel wired cages in groups of 4 per cage and kept in isolated room at controlled temperature of 22°C and ambient humidity of 65%. Lights were maintained on an artificial 12-hour light-dark cycle. Initially animals were adapted to the facility for 7 days and provided with water and standard feed ad libitum. Experimental procedures were conducted in accordance with the guide for the care and use of the laboratory animals of Institute of Marine Biology, which is following the Directive 86/609/EEC.

The whole study contained of three experiments purposed for estimation of the effects exerted by high and low-esterified pectin, and calcium pectate on (1) strontium absorption in intestine, (2) strontium removal from inner organs and bones, and (3) strontium elimination with feces.

In each experiment rats after adaptation period were randomized into 5 groups. These groups included "Control" group, which were fed the standard diet only, and 4 treatment groups, which were supposed to be administered 50 mg/kg of strontium in a form of chloride solution ("Sr", "Sr + HE pectin,""Sr + LE pectin", "Sr + Ca pectate"). The last three groups were additionally given solution/suspension containing 0.5 g/kg high-esterified pectin or low-esterified pectin or calcium pectate respectively using gastric gavage. For prevention of interaction between strontium, pectin samples and food components animals of all groups were not given access to food for 1 hour after administration of the metal solution and polysaccharide. At the end of experiment all animals were given light ether anesthesia and killed by decapitation. Liver, kidney, heart, and femora were removed, weighed, rinsed, and stored at −30°C until analysis. The strontium content in the heart, kidney, liver, and femur removed from the animals as well as in the feces was determined by atomic absorption spectrometry ("Nippon Jarrel Ash", model AA-855) according to the instruction given in the manual guide.

In the first part of the study the rats were given strontium solution and pectin preparations for three weeks for estimation of influence of polysaccharides on the metal absorption in intestine. In the second part of the study the animals were given strontium solution for three weeks and after that treated with pectin preparations for three more weeks being purposed for estimation of the metal removal velocity affected by pectin administration. In the third part of experiments the animals were also administered strontium solution for three weeks and after that treated with pectin compounds for 6 days, during which collection of feces from every rat was performed and amount of strontium in it was assessed.

Statistics

Values indicating the strontium contents in organs and bones are presented as mean ± SEM. Results were analyzed using one-way analysis of variance using Dunnett test. Differences with a value of $P < .05$ were considered statistically significant. Statistical analysis was performed using SPSS for Windows 11.0.

RESULTS

Pectin substrate

The scheme on the figure 1 illustrates the structure of the polymer pectin compounds studied in experiments. Through the chemical analysis of the pectin samples the following results were obtained. The content of pure galacturonan in the high-esterified pectin sample used in the experiments was 78.0%, degree of esterification was approximately 60.2%. The intrinsic viscosity of high-esterified pectin was 352 mL/g of galacturonan. Its molecular weight calculated using Mark-Howink equation was about 225 kDa. Low-esterified pectin was found to have the following characteristics: galacturonan concentration – 77.1%, degree of esterification – about 1.2%, intrinsic viscosity – 201 mL/g of galacturonan, calculated molecular weight – approximately 20 kDa. The free and esterified carboxyl groups of galacturonan residues in both pectin macromolecules were distributed in a random pattern. Calcium pectate sample was similar to the low-esterified pectin regarding structural characteristics but 86% of carboxyl groups in its molecule were presented in the calcified form.

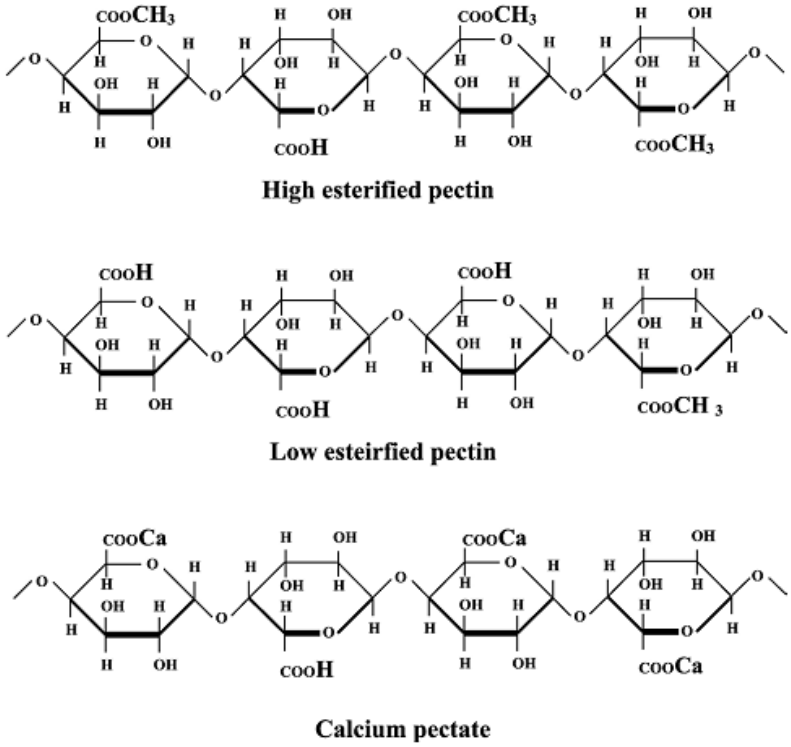

Fig. 1: Chemical structure scheme of the pectin samples studied. The main difference is the atoms attached to the carboxyl groups of galacturonic acid.

In vitro strontium binding capacity

The *in vitro* experiments showed that the highest metal binding activity in both concentrations 0.2 × 10^{-3} mol/L and 2.0 × 10^{-3} mol/L of strontium in the aqueous solution was typical of low-esterified pectin. Binding capacity of calcium pectate was 40.0% and 30.7% lower, respectively, than that of the low esterified pectin in both strontium solutions. High esterified pectin possessed lowest strontium binding capacity making up 61.4% and 44.0%, respectively, of that of the low-esterified pectin (Fig. 2).

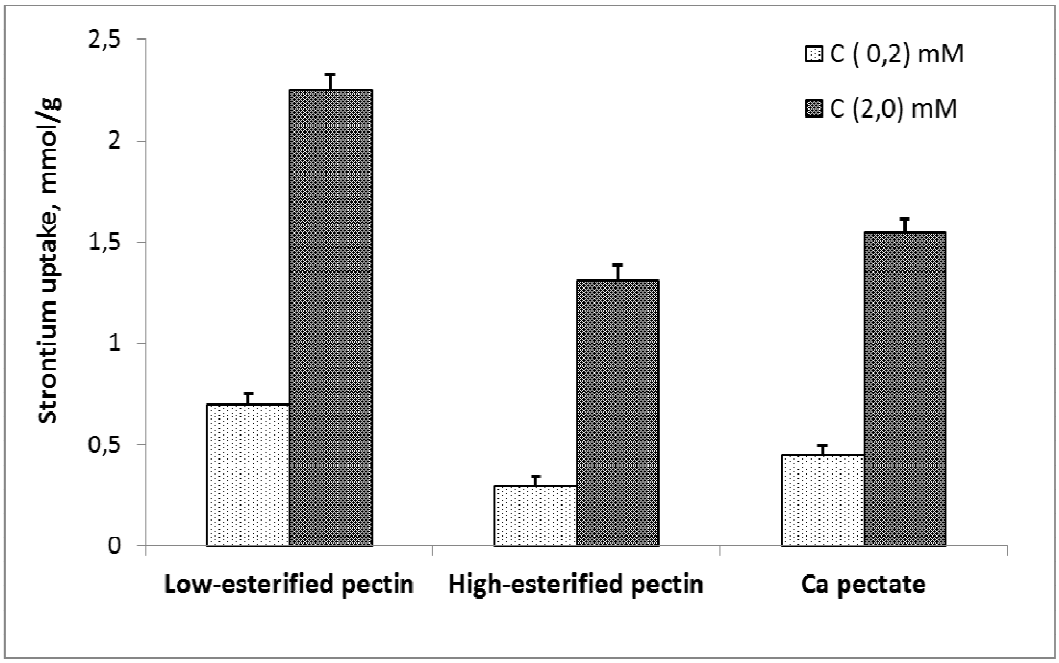

Fig. 2: Strontium binding capacity of low-esterified and high-esterified pectins and calcium pectate at different concentration of strontium in solution.

Effects of pectins on the tissue retention of strontium

As it was expected, administration of the strontium chloride in rats resulted in considerably increased accumulation of the metal in liver, heart, kidney, and femur if compared with the control group. Heart and liver were the last sensitive tissues to strontium retention; nevertheless, we found 30 and 60 times increased strontium concentration in liver and heart, respectively. Strontium content in the femur was more than 220 times higher than that in the control group (table 1). In the rats that were simultaneously given polysaccharide compounds accumulation of strontium was significantly lower. Administration of the pectin with the low degree esterification resulted in most pronounced reduction of the metal retention as in inner organs as in bones. Quantitative analysis showed that in comparison with untreated animals strontium content in heart of these rats was 2.4 times lower, in liver – also 2.4 times lower, in kidney – 2.5 times lower and in femur 1.9 times lower. Strontium amount in tissues in rats treated with calcium pectate was statistically close with those of rats given low-esterfied pectin although it may be noted that this treatment was less effective than administration of the low-esterified pectin. High-esterified pectin usage did not result in so markedly reduced accumulation of the metal in organs and bones. Nevertheless, values of strontium concentrations in inner organs as well as in femur were significantly lower in comparison with those of animals that were not treated with polysaccharide samples (Table 1).

Table 1: Strontium content in inner organs and femur in rats simultaneously administered strontium chloride and pectin compounds daily

Group	Sr^{2+} concentration (µg/g of dry weight)			
	Liver	Heart	Kidney	Femur
Control	2.3±1.3	1.8 ± 2.4	2.3 ± 1.6	3.6 ± 2.6
Sr	61.9±8.7[a]	136.2 ±12.0[a]	286.6 ± 26.3[a]	822.7 ± 23,6[a]
Sr + LE pectin	26.1 ± 3.6[a, b]	56.1 ± 6.8[a, b]	113.3 ± 12.8[a, b]	434.8 ±37.1[a, b]
Sr + HE pectin	42.7 ± 7.3[a, b]	103.7 ± 8.5[a, b]	185.8 ± 21.7[a, b]	652.4 ± 37.2[a, b]
Sr + Ca pectate	28.3 ± 3.9[a, b]	53.6 ± 7.4[a, b]	141.1 ± 19.9[a, b]	476.1 ± 76.3[a, b]

[a] Significant difference *(P* < 0.05) between "Sr" and "Control" group; [b] Significant difference *(P* < 0.05) compared with "Sr" group using Dunnett test.

Effects of pectin on strontium removal from inner organs

The results showed that administration of strontium chloride led to significant increase of strontium concentration in inner organs and bones as it was found in the first part of the study (Table 2). After next 3 weeks of experiment when strontium administration was ceased, the metal concentration in organs did not significantly change indicating that no self-healing processes had a place (Table 2). At the same time treatment of the animals with the low-esterified pectin for 3 weeks contributed to a dramatic decrease of the metal contents in the kidney and femur by 2.0 and 1.4 times, respectively. Administration of calcium pectate also resulted in decreased strontium content in the kidney and femur, although it was less pronounced in comparison with the low-esterfied pectin treatment. After the use of high-esterified pectin only amount of strontium in kidney was reduced by 1.4 times, and this reduction was considered significant. In the femur the strontium quantity did not markedly changed as a result of high-esterified pectin usage.

Table 2: Strontium content in inner organs and femur in rats given pectin samples after advance administration of strontium chloride

Group	Sr concentration (μg/g of dry weight)			
	Liver	Heart	Kidney	Femur
3 weeks				
Control	1.8 ± 0.9	2.2 ± 0.6	3.8 ± 1.1	4.1 ± 2.2
Sr	63.5±2.1[a]	139.6 ± 6.4[a]	300.2±25.8[a]	855.3 ±62.2[a]
6 weeks				
Control	1.9±0.5	2.7±1.2	3.7±1.3	4.5±1.7
Sr	64.2±5.9[a]	137.8±4.2 [a]	297.2±14.7[a]	838.8±48.6[a]
Sr + LE pectin	133.7 ± 5.1 [a,b]	206.9±9.3 [a,b]	145.0 ± 18.5 [a,b]	530.5±38.4 [a,b]
Sr + HE pectin	66.8 ± 5.3 [a,b]	152.8±3.4 [a,b]	213.7 ± 18.7 [a,b]	701.5±42.2 [a,b]
Sr + Ca pectate	137.6 ± 9.9 [a,b]	180.4 ± 6.3 [a,b]	161.5 ± 11.7 [a,b]	554.8±36.5 [a,b]

[a] Significant difference *(P* < 0.05) compared with "Control" group using Student's two-tailed test, [b] Significant difference *(P* < 0.05) compared with "Sr" group using Dunnett test.

It should be noted that in contrast, treatment with low-etseridied pectin compounds contributed to the dramatic increase of the strontium concentration in liver and heart in comparison to that in the "Control" group as well as in the "Sr" group. Maximum increase of the strontium content in these organs was registered in animals given soluble low-esterfied pectin. Administration of the high-esterified pectin resulted in no changes of the metal concentration in heart and liver; therefore, they were significantly higher than those in animals of both the "Sr" and "Control" groups (Table 2).

Effects of pectins on the strontium elimination with feces

In all groups of animals given the strontium solution increased metal concentration in feces was found indicating significant continuous strontium elimination through digestive tract. It was figured out that strontium chloride administration did result in almost 100 times risen metal content in the gastrointestinal tract within indicated period. The use of calcium pectate and, to a lesser degree, low-esteried pectin in rats previously exposed to strontium helped to significantly increase the amount of strontium being excreted with feces. The strontium elimination in the feces of rats administered calcium pectate was 56.0% higher than in untreated animals, in rats given low-esterified pectin group – 24.8% higher. Amount of strontium in feces of rats given high-esterfied pectin after the strontium exposure did not significantly differ from that of untreated animals (Table 3).

Table 3: Effect of pectin substances on the strontium excretion with feces within 6 days in rats preliminary exposed to strontium

Group	Strontium quantity, μg
Control	3.67 ± 1.39
Sr	328.54 ± 18.16[a]
Sr + LE pectin	407.34 ± 12.38[a,b]
Sr + HE pectin	351.8 ± 32.50[a,]
Sr + Ca pectate	512.62 ± 23.72[a,b]

[a] Significant difference *(P* < 0.05) in comparison with "Control" group. [b] – with "Sr" group using Dunnett test.

DISCUSSION

Environmental contamination by toxic metals including radioisotopes is a serious problem worldwide because of their incremental accumulation in the food chain and continued persistence in the ecosystem. Investigation of the possible influence of food components on the course of disorders caused by environmental pollution makes nutrition to be a key point in the public health protection. Dietary fibers have previously been studied for their potential detoxifying effect on some radioisotopes and heavy metals[19]. Pectins are the ionic plant polysaccharides widely used in food industry because of their gelling and thickening properties[20]. The main structural features of pectin are the linear chains of a-D-galacturonic acid residues, some of which are partially esterified with methanol[8]. Generally natural pectins are highly esterified, whereas low-esterified pectins can be prepared using chemical methods[21]. This study has shown that pectins bind strontium ions under in vitro conditions. The strontium binding capacity pectins is dependent on their chemical structure, particularly, degree of esterification. According to the "egg-box" model of interaction between polysaccharides and bivalent metals[12], the quantity of the metal bound to pectin is determined by the number of free carboxyl groups. In accordance to this model in our experiments pectin with the degree of esterification about 1.2% exerted highest strontium binding activity in comparison to other compounds used in the study. High esterified pectin is characterized by a major part of carboxyl group to be occupied with methyl radicals preventing interaction with the metal ions. Therefore, high-esterified pectin showed the lowest strontium binding activity. Calcium pectate possessing the same degree of esterification as the low-esterfied pectin sample was significantly less effective in vitro. It was shown previously that affinity of pectin molecule to the heavy metals is several times higher than that to calcium[10], although there are no exact data concerning pectin affinity to the strontium ions. Nevertheless, we may presume that calcium pectate may provide fast ion-exchange processes between calcium and strontium ions resulting in pronounced metal binding activity. The low strontium binding capacity of this sample under in vitro condition was caused probably by water insolubility of the calcium pectate preventing all carboxyl groups to interact with the metal ions.

During the studies estimating influence of pectin substances on the strontium toxicokinetics in rats we used the dose of strontium chloride that may be considered very high if compared to the heavy metals doses used by other investigators[22,23]. But it was suitable for modeling of severe strontium intoxication and relatively rapid accumulation of the metal in organs and bones. Strontium toxicokinetics in humans and in rats are similar[3], therefore the use of these animals are a good model for studying strontium metabolism and toxicity. The doses of pectin substances used in this study (0.5 g/kg) may be considered as close to nutritional doses because they correspond to 70 g of pectins recommended to be in a daily human diet.

The rats given strontium chloride via gastric gavage through experiments had high concentrations of the metal stored in inner organs and bones as well as eliminated with feces. Low esterified pectin and calcium pectate slowed tissue retention of strontium presumably by disrupted strontium absorption in intestine. No significant differences between effects of these substances were found. High-esterified pectin added into the strontium-enriched diet of rats did reduce metal retention in inner organs and bones but its influence may be considered as low effective in comparison with low-esterified pectin.

The use of the pectin substances in animals preliminary exposed to the high doses of strontium chloride resulted in dramatic decrease of the metal contents in kidneys and femur. Low esterified pectin and calcium pectate were similarly effective, whereas effects high-esterified pectin were less pronounced. These results suggest that administration of low-esterified pectin substances helps to eliminate strontium ions from the tissues. This presumption was confirmed by enhanced concentration on the metal in feces of rats treated with low-esterified pectin and calcium pectate.

It should be mentioned that fast elimination of the metal ions from tissues usually lead to re-distribution of the metal in the body of the animals increasing its storage in well-perfused organs. Our experiments have shown two-fold increased strontium concentration in liver and heart as a result of the pectin treatment. This phenomenon is sometimes called "rebound" effect[24]. Despite strontium redistribution may possess a danger and should be avoided during therapy with any metal-binding substance, results of our experiment confirm influence of pectin substances on strontium distribution.

As a majority of polysaccharides, pectins are heterogeneous compounds regarding structure and physicochemical properties depending on the source[25] and process of chemical and enzymatic modifications[24]. Therefore, in experiments estimating pharmacologic efficacy of different pectins it is important to have the most complete structural characteristic of the samples studied[9,26]. In our study initially we performed analytical procedures regarding mentioned structural parameters of the pectin samples used in experiments. We may conclude that strontium-removing effects are typical of the pectin substances with low degree of esterification and molecular weight about 20 kDa.

In conclusion, many chelating agents are currently used to manage heavy metal toxicity in human. The most common of them are nonspecific and have some adverse effects such as induction of microelement misbalance[27]. Because pectins is both specific and effective in complexing strontium ions, these compounds may be considered as prospective source of pharmaceutical agents that could be used for deceleration of strontium intestinal absorption, prevention of strontium accumulation, and amelioration of the strontium toxicity.

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