Strontium Inhibition of Vitamin D_3-Induced Calcium-Binding Protein (CaBP) and Calcium Absorption in Chick Intestine[1] (34604)

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In several species, high dietary strontium has been shown to produce skeletal defects analogous in many respects to vitamin D_3-deficiency rickets (1–4). On the basis of the *in vivo* and *in vitro* experiments of Sobel and co-workers (2, 5, 6), it was suggested that a direct inhibition of the calcification mechanism was the cause of the disease. The competition of strontium for specific calcium-binding sites in bone matrix was thought to be involved in preventing normal bone calcification.

The possibility that stable strontium ingestion may also interfere with the intestinal calcium absorptive mechanism was first considered by Bartley and Reber (7) who observed that the retention of oral radiostrontium was markedly reduced in pigs fed a stable strontium diet. An explanation for their observation has been made possible during the course of our studies designed to assess factors controlling the rate of synthesis of a vitamin D-dependent calcium-binding protein (CaBP) of chick intestinal mucosa. This protein appears to be intimately involved in the process of intestinal absorption of calcium (8, 9). We noted that transferring chicks raised on a normal diet to a high strontium, vitamin D_3-replete diet depressed the intestinal mucosal CaBP concentration and intestinal calcium absorption (traced with ^{85}Sr) to levels found in rachitic chicks. Prior to discovering this effect of strontium, the only known similarly drastic inhibition of these vitamin D_3-mediated responses was brought about by injection of actinomycin D, an inhibitor of DNA transcription (10). This observation of an apparently direct effect of stable strontium on the intestine, aside from its intrinsic interest, may prove to be of practical significance in the control of the hazards of fallout radiostrontium.

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TABLE I. Composition of Rachitogenic Diet.[a]

	%
Glucose	57.72
Isolated soybean protein	27.00
Corn oil	5.00
Cellulose	3.00
Glycine	0.40
DL-Methionine	0.50
Choline chloride (70% solution)	0.20
Vitamin premix[b]	1.00
Mineral premix[c]	5.63

[a] Prepared by General Biochemicals, Inc., Chagrin Falls, Ohio.

[b] Supplies the following (mg/kg of diet): niacin, 50; Ca pantothenate, 20; riboflavin, 15; thiamine HCl, 15; pyridoxine HCl, 6; folic acid, 6; menadione sodium bisulfite, 1.52; biotin, 0.6; vitamin B_{12}, 0.020; BHT, 100; vitamin A, 10,000 IU; and vitamin E, 55 IU.

[c] Supplies the following (g/kg of diet): $CaHPO_4 \cdot 2H_2O$, 20.7; $CaCO_3$, 14.8; KH_2PO_4, 10.0; NaCl, 6.0; $MnSO_4 \cdot H_2O$, 0.33; $FeSO_4 \cdot 7H_2O$, 0.33; $MgSO_4$, 3.00; KCl, 1.00; KI, 0.00267; $CuSO_4 \cdot 5H_2O$, 0.00167; ZnO, 0.00623; $CoCl_2 \cdot 6H_2O$, 0.0027; $NaMoO_4 \cdot 2H_2O$, 0.0083 and Na_2SeO_3, 0.0003.

Materials and Methods. Day-old White Leghorn cockerels were fed the modified Leach and Nesheim diet (11) described in Table I (rachitogenic) or supplemented with 4.5 IU of vitamin D_3/g diet (normal). After 2 weeks, the chicks were divided into four groups and fed the diets described in the legend to Table II. One week later, six chicks from each group were killed and particle-

TABLE II. Effect of Dietary Calcium Level and Strontium on the Calcium-Binding Activity of Chick Duodenal Mucosa.

Group	Previous diet	Experimental diet[a]	Calcium-binding activity[f] (% dose ^{45}Ca/mg of total supernatant protein)
I	Rachitogenic	Normal Ca, $-D_3$[b]	3.77 ± 0.15[g]
II	Normal	Normal Ca, $+D_3$[c]	5.99 ± 0.35
III	"	Low Ca, $+D_3$[d]	9.52 ± 0.43
IV	"	Low Ca, $+Sr$, $+D_3$[e]	4.20 ± 0.08[g]

[a] All vitamin D_3-replete diets ($+D_3$) contained 4.5 IU/g of diet. For all experimental diets, the rachitogenic diet (Table I), purchased with the calcium salts excluded, was the basal diet.

[b] Diet I: 21.29 kg of basal diet, 0.68 kg of $CaCO_3$, 0.37 kg of KH_2PO_4, and 0.38 kg of nonnutritive fiber. Diet I contained 1.2% calcium and was essentially a rachitogenic diet.

[c] Diet II: Same as Diet I except vitamin D_3-replete; essentially a normal diet.

[d] Diet III: 21.29 kg of basal diet, 0.02 kg of $CaCO_3$, 0.37 kg of KH_2PO_4, and 1.00 kg of nonnutritive fiber. Diet III contained 0.1% calcium.

[e] Diet IV: 21.29 kg of basal diet, 0.02 kg of $CaCO_3$, 0.37 kg of KH_2PO_4, and 1.00 kg of $SrCO_3$. Diet IV contained 0.1% calcium and 2.62% strontium (molar equivalent of 1.2% calcium).

[f] A tracer dose of ^{45}Ca is added to a tube containing supernatant and Chelex 100, a cation exchange resin. After mixing and centrifuging, the amount of ^{45}Ca remaining in the supernatant is proportional to the amount of binding substance (CaBP) present (8, 14). Each value is the mean $\pm$ SE; 6 chicks/group.

[g] These values were the only pair not significantly different at 1% level (Student's t test).

free supernatants were prepared from duodenal mucosal homogenates as previously described (8, 12). These supernatants were assayed for total protein by the Lowry method (13), for calcium-binding activity by an ion-exchange method (8, 14) and were electrophoresed on acrylamide gel slabs for visualization of CaBP (12). After 2 weeks on the experimental diet, intestinal calcium absorption was evaluated in five additional chicks from each group by measuring the absorption of a ^{85}Sr-labeled, calcium solution (0.3 μCi of carrier-free ^{85}Sr and 0.5 mg of calcium as $CaCl_2$/0.5-ml dose of 0.9% NaCl at pH 7.0) from a ligated duodenal segment *in situ* (10); after the 30-min absorption period, the ligated segment was removed and the entire carcass was counted in a Nuclear Chicago TOBOR large volume gamma spectrometer.

Results and Discussion. After 1 week on the experimental diets, Groups I, III, and IV chicks weighed 62, 96, and 82%, respectively, of normal (Group II) weight. These values were nearly the same for chicks fed the experimental diets for 2 weeks. Groups I and IV chicks were severely rachitic.

Table II illustrates the effect of dietary calcium level and strontium on the calcium-binding activities of duodenal mucosal supernatants. Group I supernatants, prepared from rachitic chicks, possessed only background (non-CaBP) calcium-binding activity, as expected. Group III chicks, stimulated for 1 week with the low Ca diet, possessed higher than normal (Group II) calcium-binding activity (CaBP), an adaptive response to dietary calcium deprivation that has been seen before (15). Group IV supernatants were prepared from chicks fed the low calcium–high strontium diet and, even though this diet was vitamin D_3-replete, they possessed essentially the same level of calcium-binding activity (CaBP) as the rachitic controls (Group I).

Figure 1 is a photograph of an acrylamide gel electrophoretogram of pooled supernatants from the four groups. It confirms the data reported in Table II and reveals that CaBP, although stimulated to a supra-normal concentration by the low calcium diet (Group III),

TABLE III. Effect of Dietary Calcium Level and Strontium on ^{85}Sr-Labeled, Calcium Absorption by Ligated Duodenal Segment *in Situ*.

Group	Previous diet	Experimental diet[a]	% Dose ^{85}Sr absorbed[b]
I	Rachitogenic	Normal Ca, $-D_3$	6.02 ± 1.08[c]
II	Normal	Normal Ca, $+D_3$	23.12 ± 2.35
III	"	Low Ca, $+D_3$	70.50 ± 3.22
IV	"	Low Ca, +Sr, $+D_3$	7.88 ± 0.88[c]

[a] Diets described in Table II footnotes.

[b] ^{85}Sr absorption is a direct correlate of stable calcium absorption (17). Each value is the mean ± SE; 5 chicks/group.

[c] These values were the only pair not significantly different at 1% level (Student's *t* test).

was absolutely inhibited by supplementation of the low calcium diet with strontium to the extent of the molar equivalent of 1.2% calcium (Group IV).

The absorption study, Table III, revealed that duodenal ^{85}Sr (≈ calcium) absorption exactly paralleled duodenal mucosal supernatant calcium-binding activity (or CaBP content). Vitamin D_3-mediated ^{85}Sr absorption was greatly enhanced in animals stimulated with the low Ca diet (Group III) but was depressed to the rachitic level (Group I) by high dietary strontium (Group IV). It was shown subsequently in similar but more extensive experiments (to be reported elsewhere) that dietary strontium also inhibited ^{47}Ca absorption so that the values for ^{85}Sr absorption reported here undoubtedly reflect the functioning of the calcium absorptive mechanism. The lapse of 1 week between the time of intestinal CaBP estimation and the time of measurement of ^{85}Sr absorption does not invalidate the apparent correlation between these two variables because it was later found that this correlation exists even when both variables were measured in the same animal either 1 or 2 weeks after the start of strontium feeding. The direct relationship between CaBP concentration and calcium absorption has been shown to hold in other physiological and nutritional states (15).

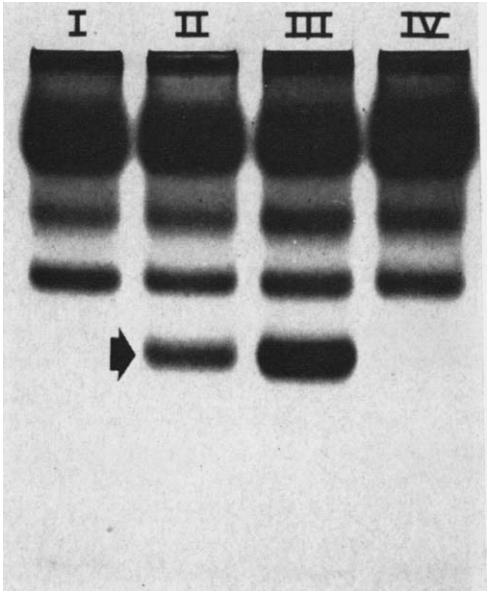

FIG. 1. Acrylamide gel electrophoretic pattern of proteins in chick duodenal mucosal supernatants. Aliquots of pooled samples were electrophoresed and contained approximately 1.7 mg of total protein. Roman numerals refer to chick groups whose diets are described in the footnotes to Table II: (I) normal Ca, $-D_3$; (II) normal Ca, $+D_3$; (III) low Ca, $+D_3$; (IV) low Ca, +Sr, $+D_3$. Arrow points to CaBP; anode at bottom.

These observations suggest that strontium-induced rickets may be due to a direct action of stable strontium on the intestinal mucosa via the synthetic mechanism for CaBP production and that the bone lesions produced by dietary strontium may be, at least in part, secondary to inhibition of the calcium absorptive mechanism. The data further suggest that the inhibitory effect of dietary strontium on CaBP and calcium absorption is perhaps qualitatively as well as quantitatively different from the effect of an equivalent level of dietary calcium.

An important implication of these studies

relates to the internal radiation hazard of fallout radiostrontium (and probably radiobarium). The efficacy of dietary strontium in markedly reducing the retention of a subsequent dose of radiostrontium has already been shown in pigs (7) and rats (16). In the study with pigs, in which the radiostrontium dose was given orally, the effect was thought to be mediated via an action of stable strontium on the intestinal absorptive mechanism. In the experiments with rats, in which radiostrontium was given intraperitoneally, the effect was thought to be due to the saturation of bone surfaces with stable strontium thereby preventing deposition of radiostrontium. The present results definitely point to the intestinal absorptive mechanism as at least one site of stable strontium inhibition of radiostrontium retention. Regardless of mechanism, however, it would seem possible to speculate that the voluntary ingestion of large quantities of stable strontium, prior to and during a period of high-level environmental contamination from fallout, might reduce radiostrontium retention to within tolerable limits. Advantageously, the skeletal defect imposed by high levels of dietary strontium may be reversible when a normal diet is again consumed (5). Before any serious recommendation in this direction can be made, however, considerably more information is needed including the effect of stable strontium on recipients of different ages, the completeness of the reversibility of the intestinal and bone defects imposed, and the timing of stable strontium ingestion in relation to the contaminating event.

In conclusion, it seems clear that the elucidation of the exact site and mechanism of stable strontium action could lead to an understanding of factors controlling the synthesis of vitamin D-induced CaBP, the role of CaBP in calcium absorption and, in addition, to a possible approach to reducing the hazards of fallout radiostrontium.

Summary. Supplementation of a low calcium, vitamin D_3-replete diet with strontium, to the extent of the molar equivalent of 1.2% calcium, induced rickets and completely inhibited CaBP production and intestinal calcium absorption after only 1 week of feeding to previously normal chicks. These observations suggest that strontium-induced rickets may be the result of interference with the calcium absorptive mechanism.

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