Proceedings
of the
Society
for
Experimental Biology and Medicine

Vol. 104 MAY, 1960 No. 1

SECTION MEETINGS

ILLINOIS	
University of Illinois	March 15, 1960
IOWA	
State University of Iowa	February 23, 1960
MARYLAND	
Johns Hopkins University	February 12, 1960
MINNESOTA	
University of Minnesota	February 25, 1960
SOUTHERN CALIFORNIA	
California Inst. of Technology	March 8, 1960

Influence of Excessive Amounts of Vit. D_3 on Strontium-89 Metabolism in the Rat.* (25705)

FRANK R. MRAZ AND JAMES A. BACON (Introduced by Homer Patrick)
University of Tennessee-Atomic Energy Comm. Agric. Research Lab., Oak Ridge, Tenn.

With the increase in use of nuclear power there is also concomitant jeopardy from fission products. Contamination of areas particularly with strontium-89 and -90 through explosions of nuclear devices and through wastes from nuclear reactors is coming into the limelight as an international problem. Hamilton(1) reported that these isotopes are available from food which might be produced on contaminated land. The primary site of deposition of strontium nuclides in the body is in the skeleton, where they are retained for long period emitting beta particles which may seriously impair the blood forming functions of bone marrow and induce bone tumors(2). Investigators have reported a clearcut reduction of strontium-90 burden with increased ingestion of calcium(3,4). Pugsley(5) observed a decrease in fecal calcium with an increase in urinary calcium during administration of irradiated ergosterol. Patrick and Bacon(6) have shown that use of Vit. D increases the efficiency of utilization of Ca^{45} and Sr^{89} in both organic and inorganic forms in the rat. This study was initiated to ascertain the effect of feeding excessive amounts of Vit. D_3 in presence and absence of dietary calcium or strontium on strontium-89 metabolism in the rat.

Methods and materials. A total of 116 weanling albino rats (Sprague-Dawley strain) were used with a minimum of 4 rats per diet in each of the experiments. The diet reported

* This manuscript is published with permission of Director of Univ. of Tennessee Agri. Exp. Station, Knoxville. Radioactive materials were obtained from Oak Ridge Nat. Lab. on allocation from U. S. Atomic Energy Comm. The work was completed under Contract between Univ. of Tenn. Coll. of Agri. and Atomic Energy Comm.

TABLE I. Strontium-89 Content of Excreta of Rats Fed Various Levels of Vit. D_3.

I.U. D_3 added to 1 kg basal diet (.5% Ca)	Excretion (%)*		
	Fecal	Urinary	Total
None	53.6	4.9	58.5
7,500	53.1	6.1	59.2
75,000	37.7	10.8	48.5
100,000	35.6	8.0	43.6
200,000	20.2	20.5	40.7
300,000	25.0	18.9	43.9
400,000	14.3	25.0	39.3
500,000	16.7	28.4	45.1
1,000,000	13.1	32.4	45.5
2,500,000	11.0	30.9	41.9
L. S. D.†	11.7	7.4	11.0

* Expressed as % of orally administered dose excreted in 72 hr.

† Least significant difference between means at 5% level of confidence.

by Hansard *et al.*(7) was used with slight modifications in the mineral mixture. Adjustments in minerals and Vit. D were made at the expense of cornstarch. Ten microcuries of strontium-89 in the chloride form were orally administered to each rat. They were then placed in metabolism units described by Hansard and Comar(8) and excreta collected daily. All samples were ashed at 600°C, dissolved in hydrochloric acid, precipitated with ammonium oxalate and counted with an end-window Geiger-Muller tube with a window thickness of 1.4 mg/cm^2. In the first experiment a Vit. D deficient diet (containing 0.5% calcium) was supplemented with levels of Vit. D_3 shown in Table I to ascertain the level necessary to produce a change in strontium-89 excretion. These diets were fed for a period of 7 days before administration of the radionuclide after which time a 72-hour balance trial was conducted. The influence of dietary calcium and strontium on excretory strontium-89 response to excessive Vit. D_3 feeding was determined in the second experiment (Table II). A calcium deficient diet was supplemented was 0.75% calcium and/or an equivalent amount of strontium (1.64%). Each of these 4 diets had a counterpart with 200,000 I.U. of Vit. D_3/kg. Rats were fed these diets for a period of 6 days before administration of strontium-89 at which time a 72-hour balance trial was initiated. In the third experiment

TABLE II. Strontium-89 Content of Excreta of Rats Fed Varying Levels of Strontium, Calcium and Vit. D_3.

Supplements to Ca-deficient basal diet	Excretion (%)*		
	Fecal	Urinary	Total
None	23.5	4.2	27.7
+ .75% Ca	60.5	3.0	63.6
+ 1.64% Sr	45.5	12.2	57.7
+.75% Ca + 1.64% Sr	64.0	13.3	77.3
+200,000 I.U. vit. D_3/kg	28.7	4.4	33.1
+ .75% Ca + 200,000 I.U. vit. D_3/kg	31.6	14.5	46.1
+ 1.64% Sr + 200,000 I.U. vit. D_3/kg	21.9	24.1	46.0
+ .75% Ca + 1.64% Sr + 200,000 I.U. vit. D_3/kg	32.8	28.7	61.5
L. S. D.†	10.2	7.4	10.0

* Expressed as % of orally administered dose excreted in 72 hr.

† Least significant difference between means at 5% level of confidence.

TABLE III. Strontium-89 Content of Excreta of Rats Fed Vit. D_3 or Dihydrotachysterol 3 Days Before or at Time of Administration of Sr^{89}.

Supplements to basal diet (.5% Ca)	Days fed before Sr^{89} admin.	Excretion (%)*		
		Fecal	Urinary	Total
None	3	28.7	3.9	32.6
Vit. D_3 (2,500,000 I.U./kg)	3	11.3	30.4	41.7
Idem	0	35.3	10.4	45.7
Dihydrotachysterol (2.5 mg/kg)	3	28.1	10.7	38.8
Idem	0	28.9	5.6	34.5
L. S. D.†		5.4	1.5	5.4

* Expressed as % of orally administered dose excreted in 96 hr.

† Least significant difference between means at 5% level of confidence.

the influence of length of period of feeding Vit. D_3 and dihydrotachysterol (a reduction product of tachysterol which is a product of irradiation of ergosterol promoting phosphaturia and mobilizing calcium from the skeleton(9)) on strontium metabolism was ascertained (Table III). Two diets were formulated from the basal diet (containing 0.5% calcium) which was deficient in Vit. D; one contained 2,500,000 I.U./kg Vit. D_3 and the other contained 2.5 mg/kg dihydrotachysterol. Three groups of rats were started on the basal diet 3 days before administration of the radionuclide and the other 2 were fed either the Vit. D_3 or dihydrotachysterol diet.

At time of administration of strontium-89, these latter diets were fed to 2 of the groups started on the basal diet. To allow more time for the effect of the diets to be shown, a 96-hour balance trial was made.

Results. Fecal excretion of strontium-89 tended to be less in diets containing more Vit. D_3 with significantly smaller amounts occurring between the 7,500 and 75,000 I.U. levels and the 100,000 and 200,000 I.U. levels of Vit. D_3 supplementation (Table I). The converse was true for urinary excretion of strontium-89 with significant increases first being shown between the 100,000 and 200,000 I.U. levels of Vit. D_3 supplementation. The increase in urinary strontium-89 was not sufficient to compensate for the decrease in fecal strontium-89 and therefore, significant decreases in total excretion of strontium-89 were apparent. While total excretion of strontium-89 was not greatly affected by massive Vit. D_3 supplementation, the amount passing through the gutwall was increased almost 5-fold as measured by fecal strontium-89. It would appear, therefore, that Vit. D_3 has the property of decreasing fecal excretion.

In the absence of dietary calcium or strontium, no significant effect on strontium-89 excretion was observed from feeding 200,000 I.U. Vit. D_3/kg of diet (Table II). However, in the diets containing calcium or strontium, the significant decreases in fecal strontium-89 and increases in urinary strontium-89 observed in the previous experiment were again shown. It would also appear that high levels of Vit. D_3 add to the effect of dietary strontium in increasing urinary and decreasing fecal strontium-89.

In every instance (Table III) inclusion of either Vit. D_3 or dihydrotachysterol significantly increased urinary excretion of strontium-89. However, the 3-day feeding period before radionuclide administration was significantly more effective in producing this effect. The level of Vit. D_3 used (2,500,000 I.U./kg) was considerably more efficient in increasing urinary strontium-89 than was the level of dihydrotachysterol used (2.5 mg/kg). While Vit. D_3 influenced fecal strontium-89, dihydrotachysterol exerted no such effect. Over these short periods, high levels of Vit. D_3 significantly increased total excretion of strontium-89.

It appears from our data that concentrations of calcium or strontium are necessary in the diet to exhibit the effect of excessive amounts of dietary Vit. D_3 on excretory pattern of strontium-89. A preliminary period of Vit. D_3 feeding before dosing was required for maximum expression of this effect. As would be expected from its effect on calcium metabolism(9), dihydrotachysterol did not appreciably influence intestinal absorption of strontium-89, but did increase its urinary excretion probably through mobilization from the skeletal pool. The increased urinary excretion of strontium-89 following Vit. D_3 feeding, however, is a reflection of its mobilization from the skeleton as well as of the increased intestinal absorption of strontium-89.

Summary. The experiments demonstrated that Vit. D_3 exerted an effect on excretion of strontium-89 by increasing absorption of strontium-89 from the gut, by decreasing fecal and increasing urinary strontium-89 providing either calcium or strontium is present in the diet. The efficiency with which Vit. D_3 exerted this effect was governed by its concentration in the diet and by length of time it was fed before administration of strontium-89.

1. Hamilton, J. G., *Radiology,* 1947, v49, 325.
2. Brues, A. M., Lisco, M., Finkel, M. P., *Cancer Research,* 1947, v7, 48.
3. MacDonald, N. S., Spain, P. C., Ezmirlian, F., Rounds, D. E., *J. Nutrition,* 1955, v57, 555.
4. Palmer, R. F., Thompson, R. C., Kornberg, H. A., *Science,* 1958, v128, 1505.
5. Pugsley, L. I., *J. Physiol.,* 1932, v76, 315.
6. Patrick, H., Bacon, J. A., *J. Biol. Chem.,* 1957, v228, 569.
7. Hansard, S. L., Comar, C. L., Plumlee, M. P., PROC. SOC. EXP. BIOL. AND MED., 1951, v78, 455.
8. Hansard, S. L., Comar, C. L., *Nucleonics,* 1953, v11, 44.
9. Albright, F., Bloomberg, E., Drake, T., Sulkowitch, H. W., *J. Clin. Invest.,* 1938, v17, 317.

Received February 19, 1960. P.S.E.B.M., 1960, v104.