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THE INFLUENCE OF THE VITAMIN D AND PHOSPHATE CONTENT OF THE DIET ON CALCIUM AND STRONTIUM ABSORPTION FROM THE GASTROINTESTINAL TRACT

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The effect of various amounts of phosphates and vitamin D in the diet on calcium and strontium absorption was studied in rats. The animals were kept on experimental diet for 6 days and during that period they received radioactive isotopes of calcium (^{45}Ca) and strontium (^{85}Sr) in drinking water. Skeletal retention of radioactive calcium and strontium was determined.

Increased dietary phosphates (from 0.5 to 1.2% P) were found to cause a reduction in the retention of radioactive calcium and strontium after oral administration of isotopes. The effect of phosphates proved to be much stronger on strontium than on calcium absorption. This is in agreement with our earlier results. The effect of phosphates on calcium and strontium metabolism proved independent of the vitamin D content in the diet if this content ranged from 200 to 800 I. U. per 100 g of dry food.

Vitamin D is known as an important factor in the regulation of the homeostasis of calcium metabolism in the body. Numerous literature data confirm the fact that vitamin D increases strontium and calcium absorption from the gastrointestinal tract (1, 2) as well as their elimination in the urine (1, 3). In our earlier studies we found that by varying the phosphate content of the diet within physiological limits it is possible to considerably influence the comparative metabolism of calcium and strontium (4). It seems that phosphates act by reducing strontium and calcium absorption from the gut as well as by diminishing their elimination by the kidney (5).

The purpose of this study was to establish whether small physiological variations in the vitamin D content in the diet are going to affect the action of phosphates on calcium and strontium absorption.

The results obtained indicate that this effect of phosphates on calcium and strontium absorption is independent of smaller variations in the vitamin D content in the diet.

METHODS

Female albino rats aged 11–12 weeks were used in experiments.

The animals were fed 6 special diets with varied phosphate and vitamin D content – three with a lower (0.5% P) and three with a higher phosphate content (1.2% P). The diet with a given content of phosphorus was divided into 3 subdiets with regard to the vitamin D content which was 200, 600 and 800 I. U. per 100 g of dry food.

Diets were prepared by removing bone and fish flour from the normal rat diet of »Vetserum« production, and adding instead the inorganic salts of calcium and phosphorus as calcium chloride and potassium dihydrogen phosphate. Beside, in all diets plain edible oil was used instead of cod-liver oil, to which the necessary amount of vitamin D were added (»Pliva« D oil containing 20.000 I. U. of vitamin D per 1 ml.). The amount of 200 I. U. of vitamin D/100 g of dry food corresponds to the amount of vitamin D usually added to the standard »Vetserum« diet as cod-liver oil. All animals received food ad libitum.

To follow the absorption of calcium and strontium radioactive isotopes ^{45}Ca and ^{85}Sr were applied to rats orally in doses of 0.3 μCi in 10 ml of distilled drinking water.

During the experiment animals were kept in individual metabolic cages in which it was possible to measure the quantity of the water consumed and thus to determine the amount of administered dose of the radioisotopes. At the end of experiments rats were sacrificed. Skeletons were dissected and mineralized in a muffle furnace at 800 °C. The mineralized skeleton was then dissolved in hot hydrochloric acid and the sample was made up to volume with distilled water.

The activity of ^{85}Sr was assessed in the scintillation counter (EKCO). To determine ^{45}Ca preliminary radiochemical treatment of the samples was necessary including the precipitation of calcium and strontium as oxalates and plating of samples for assessment of activity in a GM end-window counter. The necessary corrections for self-absorption and radioactive decay were performed.

Table 1

The influence of the vitamin D and phosphate content of the diet on calcium and strontium absorption from the gastrointestinal tract (oral administration of ^{85}Sr and ^{45}Ca)

Group	^{85}Sr				^{45}Ca			
	Skeletal dose %							
	No. of rats	Diet 0.5% P	No. of rats	Diet 1.2% P	No. of rats	Diet 0.5% P	No. of rats	Diet 1.2% P
I	8	11.3±1.15	8	5.4±0.36	8	24.3±1.27	8	23.9±1.31
II	8	8.9±0.75	7	5.8±0.71	8	22.0±0.79	8	18.8±1.56
III	8	10.7±0.79	7	7.1±0.49	8	28.6±1.32	7	22.8±1.37

I = 200 I. U. D vitamin/100 g of dry food

II = 600 I. U. D vitamin/100 g of dry food

III = 800 I. U. D vitamin/100 g of dry food

RESULTS

Animals were divided in 6 groups of 8 rats each. Each group was fed different diet as presented in Table 1. Animals were on experimental diets through 6 days. In the course of the third and fourth day they were given radioactive isotopes of calcium and strontium in drinking water. They were sacrificed 48 hours later.

The results of skeletal retention of radioactive isotopes are presented as percentage of the consumed dose. The result of each group is presented separately as the arithmetic mean with the standard error of the mean.

From Table 1 it is seen that the skeletal retention of ^{85}Sr in rats fed lower phosphorus diet was considerably higher than the retention of strontium in the group of animals fed higher phosphate diet. This is in agreement with our previous results (4, 5). A four-fold increase of the vitamin D content in the diet (from 200 to 800 I. U.) did not essentially influence this effect of phosphates.

The effect of phosphates on skeletal retention of radioactive calcium was considerably less intense. This effect of phosphates was also independent of the vitamin D content in the diet.

Different effect of phosphates on strontium and calcium metabolism is also evident from the $^{85}Sr/^{45}Ca$ ratios in the skeleton of the rat (Table 2). The $^{85}Sr/^{45}Ca$ ratio in the rats fed higher phosphate diet is lower than the same ratio in the group of animals on a lower phosphate diet indicating increased discrimination against strontium. This effect of phosphates proved equally independent of the vitamin D content in the diet.

Table 2

The influence of the vitamin D and phosphate content of the diet on $^{85}Sr/^{45}Ca$ ratio in the skeleton (oral administration of ^{85}Sr and ^{45}Ca)

Group	No. of rats	Diet 0.5% P	No. of rats	Diet 1.2% P
I	8	0.469±0.029	8	0.231±0.008
II	8	0.396±0.025	7	0.307±0.041
III	8	0.374±0.031	7	0.322±0.015

I = 200 I. U. D vitamin/100 g of dry food
II = 600 I. U. D vitamin/100 g of dry food
III = 800 I. U. D vitamin/100 g of dry food

DISCUSSION

Although vitamin D represents a significant factor in the regulation of calcium metabolism, it is not surprising that under our experimental conditions vitamin D produced no significant effect on calcium and strontium metabolism. It is known that rats grow when fed diet with the normal content of calcium and phosphorus and that the process of calcification is going on even in the absence of vitamin D (6). However, if the mineral intake is low or the Ca:P ratio is disturbed, the effect of vitamin D becomes more apparent (7). The purpose of our experiments was therefore not to prove whether vitamin D affects calcium and strontium metabolism – for this has been sufficiently dealt with in literature but to find out whether small variations in the vitamin D content in the diet within physiological limits will affect the already established effect of phosphates on calcium and strontium metabolism. This finding is important for a possible practical application of our earlier findings concerning the effect of phosphates on calcium and strontium metabolism.

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Sadržaj

UTJECAJ SADRŽAJA D VITAMINA I FOSFATA U HRANI NA APSORPCIJU KALCIJA I STRONCIJA IZ PROBAVNOG TRAKTA

Istraživali smo djelovanje različitih količina fosfata i D vitamina u hrani štakora na apsorpciju stroncija i kalcija iz probavnog trakta. Štakori su 6 dana bili na određenom dijetnom režimu, i u to su vrijeme primali radioaktivne izotope kalcija i stroncija (^{45}Ca i ^{85}Sr) oralnim putem. Odredili smo retenciju radioaktivnog kalcija i stroncija u skeletu.

Povišeni sadržaj fosfata u hrani (od 0.5 na 1.2% P) izaziva sniženje apsorpcije radioaktivnog kalcija i stroncija iz probavnog trakta. Fosfati djeluju znatno jače na metabolizam stroncija negoli kalcija. Ti su nalazi u skladu s našim ranijim rezultatima. Spomenuti efekti fosfata na metabolizam kalcija i stroncija nezavisni su od količine D vitamina u hrani ukoliko ona raste od 200 do 800 i. j. na 100 g suhe hrane.

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